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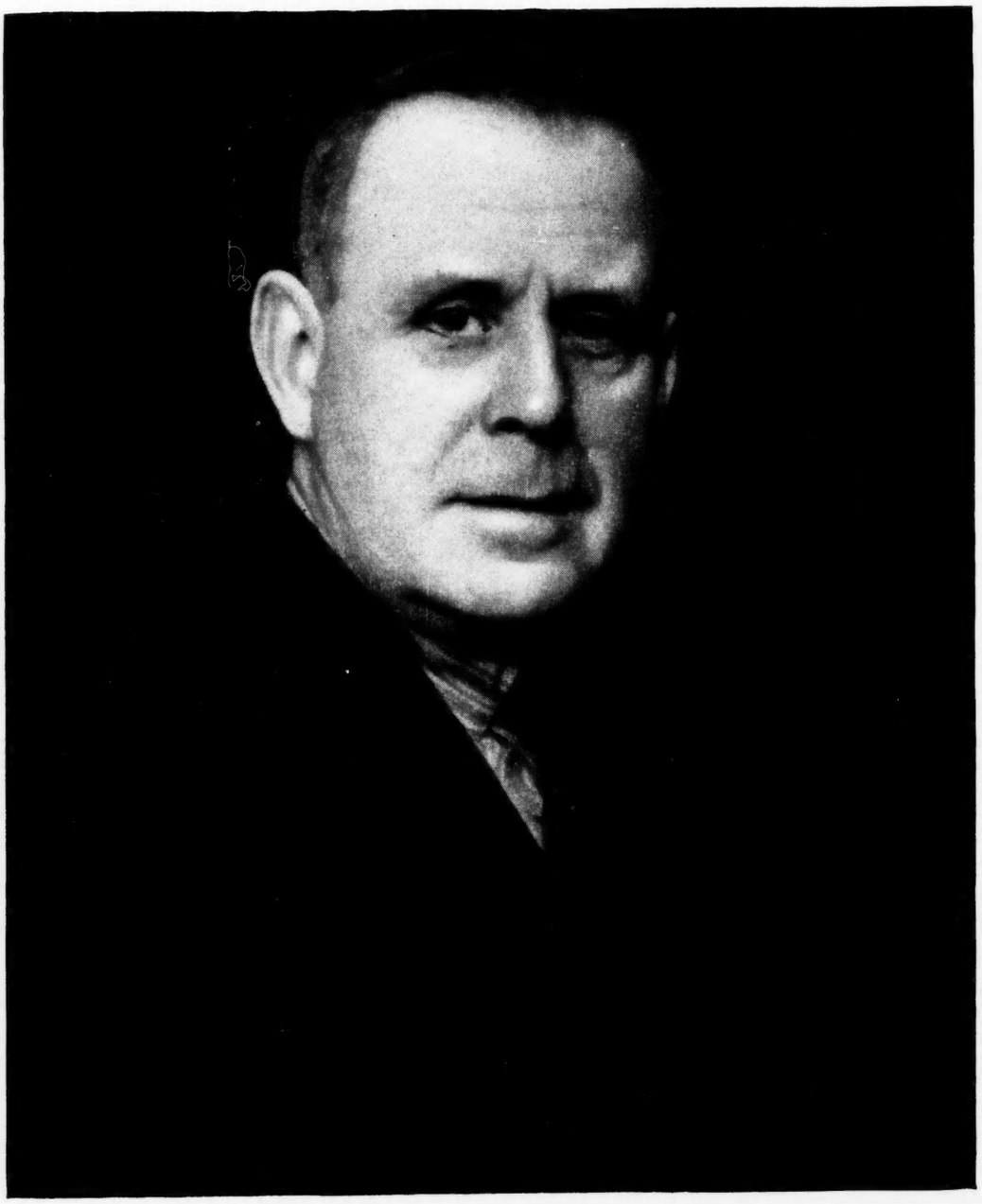

T. Southwell.

THE DISSOCIATION CONSTANTS OF PLASMOQUINE

BY

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AND

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(*Received for publication November 10th*, 1939)

In a previous paper by one of us (Christophers, 1937) determinations were given of the dissociation constants of quinine and of atebrin. The present paper deals with similar determinations for plasmoquine.

We attach importance to the determination of these values for anti-malarial compounds since their dissociation constants largely determine their reactions and are useful to know in practice. There is a further importance, however, in the possible relation of the possession of basic properties by these substances to the rationale of their therapeutic action. The possession of basic properties arising from the existence of basic groups in the molecule is a character common alike to the natural cinchona alkaloids, to effective derivatives of these, such as hydroquinine, and to synthetic compounds, such as plasmoquine and atebrin, possessing anti-malarial properties. Schulemann (1932), speaking of the steps in the discovery of plasmoquine—the first synthetic anti-malarial drug to be produced—notes that it was only on introducing a basic side-chain that efforts to obtain an effective compound were successful. If, however, the possession of basic properties is a feature necessary to the therapeutic effectiveness of these substances, then such properties must be of a certain order, i.e., the dissociation constants relative to them must lie within a certain range of value. If the constant be below a certain value, the molecular group concerned will, at the pH prevailing in the body, be entirely undissociated and hence inoperative in this respect. Again, groups having a constant above a certain value would be completely dissociated and hence possibly ineffective on this account. The natural cinchona alkaloids have constants compatible with such considerations. Of synthetic compounds only the dissociation constants of atebrin have so far been determined. But these again are compatible with the view that a certain degree of basicity is necessary. In ascertaining the constants for plasmoquine we obtain data for another synthetic compound and one which has a special interest on account of its known gametocytocidal properties.

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*Leverhulme Fellow under the Medical Research Council.
†Work carried out under the Medical Research Council.

All the anti-malarial compounds of the type under discussion, so far as is known, possess two dissociation constants, of which one is relatively strong, the other weaker. In the case of quinine the weaker constant (k_2) would appear to be of negligible importance in the body, the stronger constant (k_1) being that with a more probable relation to dissociation under such conditions. In atebrin it is the weaker constant (k_2) which more nearly approaches the body pH. It is by no means certain, however, that the possession of two constants—a feature present in all these drugs—may not have some significance.

It is perhaps desirable, in connection with our work, to point out the relation of the ' true ' to the ' apparent ' constant in the case of certain organic bases. In such substances there may exist under the appropriate conditions any three of the following: (1) the unhydrated base, (2) the hydrated but undissociated base, and (3) the dissociated base, each having their own solubilities in water, ether, etc. The amounts of these three substances present under any given conditions are determined, in the case of (1) and (2) and of (2) and (3), by distinct and separate reactions. The reaction between (1) and (2) (hydration) proceeds in accordance with the hydration constant, that between (2) and (3) (true dissociation constant) in accordance with the dissociation constant. In practice, however, it is the relation between (1) and (3) that is most readily determined (apparent dissociation constant). The true constant can only be arrived at when the hydration constant is known. In the case of the amines the true and apparent constants may be widely different, owing to the large amount, in some cases, of unhydrated base. How far such considerations apply to substances like quinine, atebrin and plasmoquine is, we believe, unknown. In the case of the amines the only method referred to in the literature for determining the true constant is by observations at three equally spaced temperatures, involving the determination in each case of the ether-water coefficient and the apparent dissociation constant (Moore, 1907; Moore and Winmill, 1912; see also Sidgwick, 1937). We have attempted to carry out such observations with plasmoquine, using an apparatus in which potentiometer and other observations could be carried out at 10°, 20° and 30° C. The difficulty of satisfactorily determining the ether coefficient for plasmoquine (referred to later in this paper) prevented any useful result being obtained from this attempt.

I. MATERIALS AND METHODS

The plasmoquine employed was a pure specimen of plasmoquine dihydrochloride supplied to us by Bayer and Co., through the kind services of Dr. F. M. Peter, to whom we are very greatly indebted.

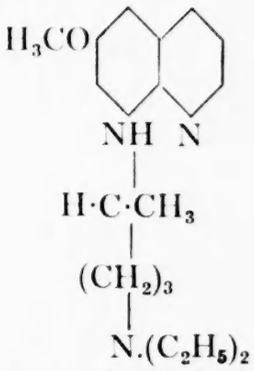
The pH determinations were made with the glass electrode, Morton's apparatus being used in the manner previously described by one of us. Observations, unless otherwise stated, were carried out at 20° C. The water was thrice distilled and freed from CO_2 by boiling. Molar concentrations are used throughout the paper unless otherwise stated.

The usual method adopted for fixing points on the titration curves was to determine the pH of mixtures containing a known concentration of the plasmoquine salt (usually 0·0002) and varying quantities of added NaOH to a fixed total volume of fluid. These mixtures were made with freshly boiled water and with precautions to minimize as much as possible exposure to the air. Titrations were, however, also made in the usual way by repeated additions of known amounts of 0·02 NaOH to a known original volume of plasmoquine solution. It was necessary in this case to make allowance in the calculations for the increase in volume thus brought about.

For that portion of the curve intermediate between the two constants, where very small additions of NaOH produced large shifts in pH, many observations were made, the results given being the means of these. Some difficulty was found (as occurs also with quinine and with atebrin) in titrating in the region of the k_1 curve, as the bulb requires to be allowed some considerable time to register a final value. This has made determinations within this range less satisfactory than those carried out in the more acid mixtures of the k_2 portion of the curve.

II. CONSTITUTION

The constitution of plasmoquine as given by Schulemann, Schönhöfer and Wingler (1932) is as shown below.



Three N atoms are present, each of which might conceivably give rise to a dissociation constant. But, as with atebrin, only two constants are demonstrable. The stronger constant (k_1) as determined by us has a value very near that of the stronger constant in atebrin and is probably connected with the diethylamino group of the side-chain.

The weaker constant (k_2) of plasmoquine would seem, most probably, to be associated with the substituting amino group attached at position 8 in the quinoline molecule, but presumably might be derived from the N atom in the quinoline ring itself. We have found that 6-methoxy-8-amino-quinoline, without side-chain, forms in aqueous solution only a monohydrochloride, combining

with only one equivalent of acid. Titrations carried out with this substance gave a dissociation constant (apparent) of approximately $k = 10^{-10}$. Quinoline, according to Kolthoff (1925), has a constant of $10^{-9.5}$. It appears difficult therefore, on *a priori* grounds, to decide to which N atom the weaker constant, as determined by us for plasmoquine, is to be attributed. If associated with the substituting amino group it is much weaker than the constant we have ascribed to this group in atebrin. On the other hand, it is very similar to the second constant in quinine, where no question of any other group than the quinoline N has to be considered.

III. DETERMINATION OF THE CONSTANTS BY TITRATION

In fig. 1, A, is shown the graph obtained by varying amounts of 0·02 NaOH in mixtures with a total concentration of 0·0002 plasmoquine. There is a marked inflection in the neighbourhood of the addition of one equivalent of NaOH, i.e., where the curve for k_2 changes to that for k_1. In the same figure is shown (B) the titration curve in the region of the k_2 constant for a stronger solution (0·0036). Here the inflection is still more marked, with an almost horizontal portion of curve. The reason why curve B is not completed is that in such concentration precipitation of the base prevents observations being carried beyond the point given in the graph.

As will be seen from graph A, the curves for k_1 and k_2 are relatively widely separated, so that little disturbance by one constant is evidenced in the curve for the other. Determination of the constants by titration should therefore offer no special difficulty. With certain reservations this was the case, and, since determination of the constants by the ether method, as explained later, was not found satisfactory, our most reliable results are those obtained by titration.

Second Dissociation Constant

Half neutralization point. From inspection of the curve A in fig. 1 a value of pH 4·14 (pOH 9·92) would appear to be given at the half neutralization point with addition of half equivalent of NaOH. In curve B, with a more concentrated solution, a lower pH is indicated. These results, however, require considerable modification in order to arrive at the constant, since there is a very high hydrolysis effect which necessitates correction by calculation.

Calculation of constant from titration values. Calculation of k_2 from the data obtained, making allowance for hydrolysis, is given in Table I. The calculation has been based on the following considerations and the formula derived from these is given below.

A solution of the dihydrochloride before the addition of any NaOH shows a certain $[H^+]$. This is approximately equal to the concentration of free acid liberated as the result of hydrolysis. But, since hydrolysis of the salt consists in conversion of a portion of the di-salt into equal proportions of free HCl and

mono-salt (which latter in the present connection counts as undissociated component), there is in such a solution a concentration of mono-salt equal in amount to that of the free acid. NaOH, being a strong electrolyte, will at first be used

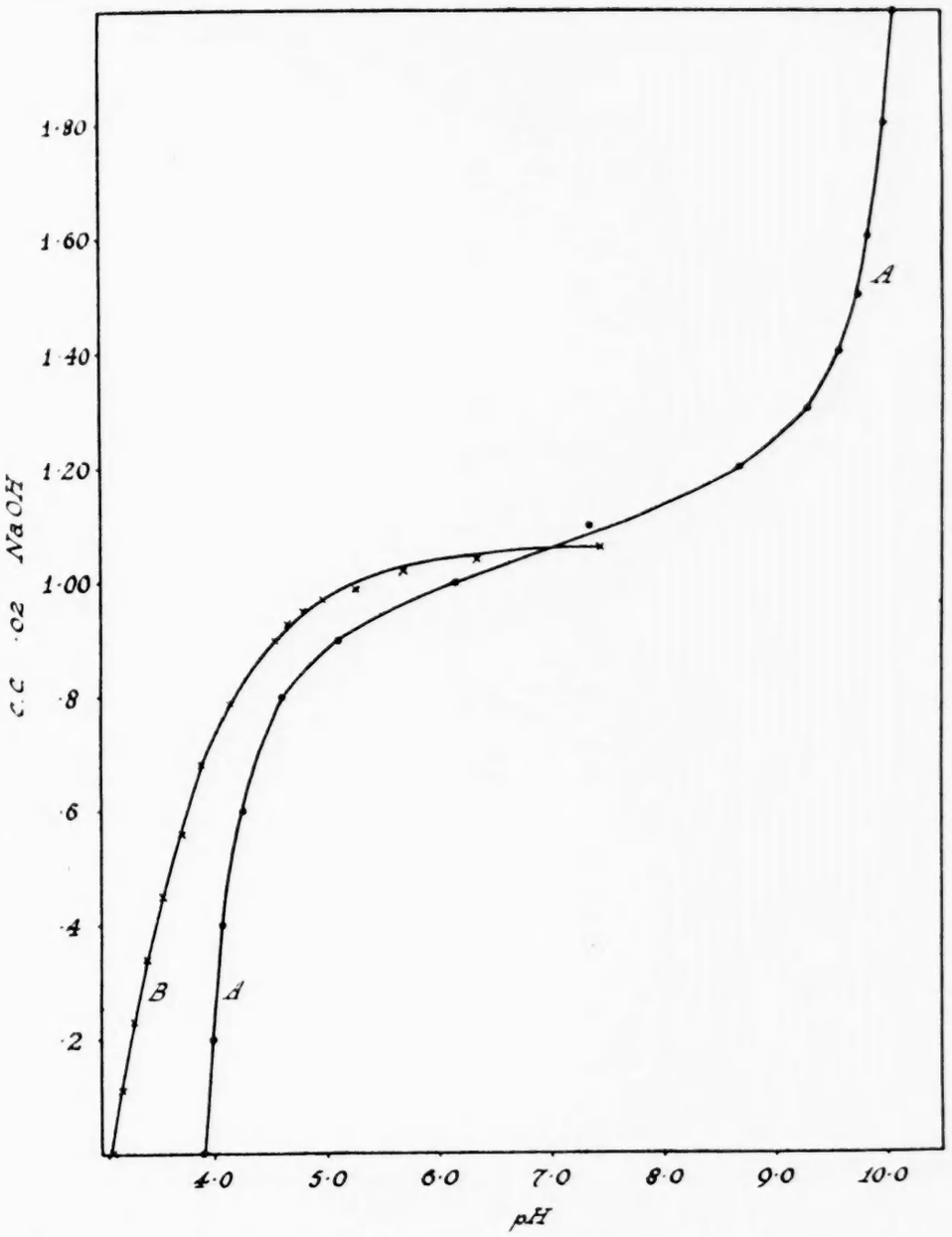


FIG. 1. Showing titration curves for plasmoquine. For explanation, see text. The scale of c.cm. indicates the amount of 0·02 NaOH in mixtures having a total volume of 100 c.cm. and refers to the curve A (0·0002 plasmoquine). Equivalent quantities (i.e., 18 times greater) were made as additions in titration of 50 c.cm. 0·0036 plasmoquine in the case of curve B.

up in neutralizing this free acid. This fact probably accounts for the character of the curve for k_2 at its acid end, which resembles a dilution curve rather than a dissociation curve. As increasing amounts of NaOH are added these will

therefore be neutralized, forming NaCl, but there will also be present (1) a concentration of mono-salt equal to that of the NaOH neutralized (i.e., to the added amount), and (2) a further amount of mono-salt proper to the hydrolysis of such di-salt as would be left unchanged by such addition of NaOH. The proportion (f) of dissociated to undissociated component is therefore given by the following expression (as used in the calculations in Table I), where M is the original total concentration of plasmoquine, N the concentration of added NaOH, $[H^+]$ the hydrogen ion concentration, and f the fraction $\frac{B^{++}}{B^+}$:

$$f = \frac{M - N - [H^+]}{N + [H^+]}$$

The effect of $[OH^-]$ in this range in calculating f is too small to be shown in the calculation.

From the value f and the observed pH the constant can be ascertained from the formula

$$pk_2 = pOH - \log f$$

pOH being obtained by subtracting the observed pH value from the ionization constant of water for the temperature (20° C.), taken in this case as 14·06.

Neglecting a few outlying values in a part of the curve where accuracy is difficult, the values are given of 10·55 and 10·51 in A and B respectively, a somewhat lower value being given in A if all observations are included and a somewhat higher value in both A and B if observations are confined to those near the point of half neutralization.

Plotted in the form of a dissociation curve against α, the observations fall as shown in the right-hand curve in fig. 2.

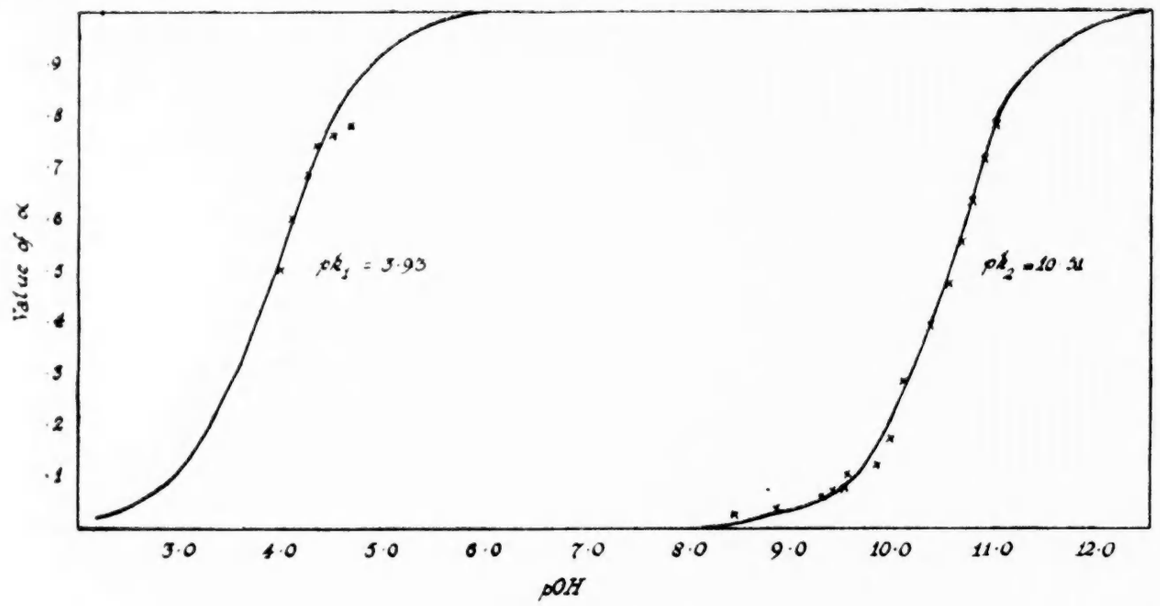


FIG. 2. Showing titration values plotted as dissociation curves. a, the dissociated fraction, is given by f/1+f.

TABLE I

Showing calculation of k_2 from data given by titration of plasmoquine dihydrochloride with NaOH. For explanation of column headings, see text

M $\times 10^6$	N $\times 10^6$	M–N $\times 10^6$	pH	pOH	$[H^+]$ $\times 10^6$	M–N–$[H^+]$ $\times 10^6$	N+$[H^+]$ $\times 10^6$	f	log f	pk_2	α
A. Total concentration of plasmoquine 0·0002 (mixtures to same total volume)											
200	0	200	3·90	10·16	126	74	126	0·59	−0·33	**10·39**	0·37
..	40	160	3·98	10·08	105	55	145	0·38	−0·42	**10·51**	0·28
..	80	120	4·07	9·99	85	35	165	0·21	−0·68	**10·66**	0·17
..	120	80	4·25	9·81	56	24	176	0·14	−0·80	**10·66**	0·12
..	160	40	4·60	9·46	25	15	185	0·08	−1·09	**10·55**	0·07
..	180	20	5·10	8·96	8	12	188	0·06	−1·19	10·15*	0·06
..	188	12	5·42	8·64	3·8	8·2	192	0·04	−1·37	10·01*	0·01
..	196	4	5·91	8·15	1·2	2·8	197	0·01	−1·85	10·00*	
..	200	0	6·12	8·15							
						MEAN (excluding results marked with asterisk)				**10·55**	
B. Total concentration of plasmoquine 0·0036 (titration 50 c.cm. with 0·02 NaOH)											
3600	0	3600	3·10	10·96	790	2810	790	3·56	0·55	**10·41**	0·78
3529	392	3137	3·19	10·87	646	2491	1038	2·40	0·38	**10·49**	0·71
3461	769	2692	3·29	10·77	513	2179	1282	1·70	0·23	**10·54**	0·63
3396	1132	2264	3·40	10·66	398	1866	1530	1·22	0·09	**10·65**	0·55
3333	1481	1852	3·54	10·52	288	1564	1769	0·88	−0·06	**10·60**	0·47
3273	1818	1455	3·71	10·35	195	1260	2013	0·63	−0·20	**10·55**	0·39
3214	2143	1071	3·90	10·16	126	945	2269	0·42	−0·38	**10·54**	0·30
3158	2456	702	4·14	9·92	72	630	2528	0·21	−0·68	**10·60**	0·17
3103	2759	344	4·54	9·52	29	315	2788	0·11	−0·96	**10·48**	0·10
3093	2818	275	4·66	9·40	22	253	2840	0·09	−1·05	**10·45**	0·08
3082	2877	205	4·80	9·26	16	189	2893	0·07	−1·19	**10·45**	0·06
3072	2935	137	4·98	9·08	10	127	2945	0·04	−1·37	**10·45**	0·04
3061	2993	68	5·27	8·79	5	63	2998	0·02	−1·68	**10·47**	0·02
3051	3051	0	5·69	8·37	2						
3041	3108		6·32	7·74							
3030	3165		7·42	6·64							
									MEAN	**10·51**	

By degree of hydrolysis. k_2 is also deducible from the extent of the hydrolysis according to the following formula for hydrolysis of the di-salt (see Christophers, 1937, formula (2)):

$$pH = 7 - \tfrac{1}{2}pk_2 - \tfrac{1}{2}\log C$$

where C is the total original concentration of the salt. The following are deter-

minations of the pH at different concentrations of the di-salt, with the value for pk_2 as calculated from the formula. The *degree of hydrolysis* $[H^+]/C$ is also given.

C	pH	pk_2	$[H^+]/C$
0·0001	4·20	9·60	0·63
0·0002	3·90	9·90	0·63
0·0004	3·64	10·12	0·57
0·001	3·35	10·30	0·45
0·004	2·94	10·52	0·29

It will be observed that a solution of the salt of concentration 0·0004 or below is more than half mono-salt with a corresponding concentration of free HCl. The figures may be compared with those previously given (Christophers, 1937) for quinine dihydrochloride. Hydrolysis is of the same order as for the quinine salt, but somewhat greater, in accordance with the slightly smaller dissociation constant for plasmoquine.

The value of the (apparent) constant as obtained by titration. A figure of 10·51 has been taken as the value of pk_2 for plasmoquine as shown by titration. In comparing this with the value of 9·85 previously given for quinine (Christophers, 1937) it should be noted that no allowance in the case of quinine was made for hydrolysis. The solutions of quinine used were, however, much more concentrated and the effect due to hydrolysis in this case was therefore much smaller. If allowance for hydrolysis be made on the data given, the corrected figure would be 9·86.

First Dissociation Constant

Half neutralization point. The half neutralization point as indicated in fig. 1, graph A, is at pH 9·75, giving a value for pk_1 of 4·31, taking the ionization constant for water as 14·06. If allowance be made for the high value of $[OH^-]$ this figure, as shown in the calculations given in Table II, requires some modification.

Calculation of constant from titration values. In Table II are given calculations of pk_1 from data obtained in this portion of the titration curve for a solution of concentration 0·0002 plasmoquine. In making the calculations we have used the formula given below, where M and N have the same significance as when calculating pk_2, and the same applies to f and α.

$$f = \frac{M - N + [OH^-]}{N - [OH^-]}$$

Excluding the first three outlying values, the mean figure given is 3·93.

Plotted against α the observations have the relation to the usual dissociation curve shown in the left-hand curve of fig. 2.

Determination of the equivalence point. We have endeavoured to determine with some accuracy the equivalence point in the titration curve where, as already noted, a sharp inflection is made between the curves for the two constants. The importance of this point lies in the fact that it is expressed by the relation given below for the temperature at which the observations were made :

$$pH = 14{\cdot}06 - \tfrac{1}{2}(pk_1 + pk_2)$$

Owing to the large change in pH produced by addition of very small amounts of NaOH in this region, we have not been able to arrive at such precise determination as was desired, but a considerable number of titrations indicated the greatest change in pH as occurring on either side of pH 7·0. With this value, and taking pk_2 as 10·51, the figure for pk_1 works out as 3·62, but a pH reading of 6·8 would have given a result similar to that in the titration.

TABLE II

Showing calculation of k_1 from data given by titration of plasmoquine dihydrochloride with NaOH. For explanation of column headings, see text. Total concentration of plasmoquine 0·0002 (mixtures to same total volume)

M $\times 10^6$	N† $\times 10^6$	M–N $\times 10^6$	pH	pOH	$[OH^-]$ $\times 10^6$	M–N+ $[OH^-]$ $\times 10^6$	N– $[OH^-]$ $\times 10^6$	f	log f	pk_1	α
200	0	200	6·12	7·94	0·01	200	0				
..	20	180	7·32	6·74	0·18	180	20	9·0	0·95	5.79*	0·90
..	40	160	8·70	5·36	4·4	164	36	4·6	0·66	4·79*	0·82
..	60	140	9·30	4·76	17·4	157	43	3·7	0·56	4·20*	0·78
..	80	120	9·56	4·50	32	152	48	3·2	0·50	**4·00**	0·76
..	100	100	9·75	4·31	49	149	51	2·92	0·47	**3·84**	0·74
..	120	80	9·81	4·25	56	136	64	2·13	0·33	**3·92**	0·68
..	160	40	9·97	4·09	81	121	79	1·53	0·18	**3·91**	0·60
..	200	0	10·06	4·00	100	100	100	1·00	0·00	**4·00**	0·50
									MEAN (excluding results marked with asterisk)	**3·93**	

†Exclusive of the amount of NaOH, equivalent to 200 on this scale, required to complete the k_2 titration.

The value of the (apparent) constant as obtained by titration. The constant appears to be below 4·00 and has been taken as 3·93, the value given in calculation of the titration data. This is very much higher than the value for the first constant for quinine (5·70) previously given by one of us, but is not far removed from that for atebrin (3·88).

IV. DETERMINATION OF THE CONSTANTS BY OTHER METHODS

The very marked difference between the two constants of plasmoquine is as marked a feature of this drug as is the very small interval between the two in atebrin. This wide separation of the constants not only made it less essential to utilize the ether method, but actually made this method more difficult of application, since mixtures must usually have very small relative amounts of one or other of the two forms of base. As it was thought, however, that use of the ether method, as employed by one of us (Christophers, 1937) in determining the constants of atebrin, might give some further information, it was decided to try it. Our results, however, were very disappointing. In the first place, in spite of a considerable amount of work, we found it very difficult to get satisfactory values for the water-ether coefficient for plasmoquine. The fact that plasmoquine could not be accurately determined in the small amounts present was a contributory cause. A further difficulty was found in determining satisfactorily the titratable base, as the pH to which the titration should be carried was only approximately known, the curve at this point being very steep, as will be seen from fig. 1. Lastly, on account of the wide separation of the constants it was difficult to hit off suitable mixtures. Under these conditions, as our results had no critical value and we have been unable to continue our observations further in this direction, it appears to us unnecessary to give any further details. Conduction experiments have not formed part of the line of observations undertaken by us. For the present, therefore, our observations on plasmoquine must be restricted to those we have given relating to titration.

SUMMARY AND CONCLUSIONS

1. Plasmoquine possesses two demonstrable dissociation constants: one, probably connected with the diethylamino group of the side-chain, having a value as determined by us of 3·93; the other, much weaker and of uncertain origin, determined by us as 10·51.

2. These determinations have been made by titration of suitable concentrations of plasmoquine dihydrochloride. Determinations by the ether and other methods were found difficult, owing partly to the difficulty of determining satisfactorily the water-ether coefficient of plasmoquine, and partly to the wide separation of the constants and other causes.

3. The constants as determined must be considered as the apparent constants, and under the circumstances noted in Section IV above it was found impracticable to carry out observations necessary to determine the true constants by the method of Moore and Winmill (1912).

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THE GENERA OF CERATOPOGONIDAE

BY

J. W. S. MACFIE

(*Received for publication November 20th*, 1939)

Since Kieffer's key (1926) was published, no other attempt has been made by anyone particularly interested in the family to set out the means for the determination of all the genera of the Ceratopogonidae, although several partial keys have been constructed, notably those of Johannsen (1932) for the Malayan subregion, and Goetghebuer (1933) for the Palaearctic region. Although published in 1926, Kieffer's key was written several years earlier—in September, 1922. Many new genera have been described since that date, and the need for a new survey of the family has become evident. I have therefore attempted in the pages which follow to show how all the known genera may be recognized, and to indicate the groups into which they may be separated. In doing this I have concerned myself with the extant forms only, and have not taken into consideration the fossil species found embedded in amber.

I have to thank Dr. F. W. Edwards not only for furnishing particulars about some specimens in the British Museum collection, but also for valuable criticism and suggestions on several difficult or doubtful points.

CERATOPOGONIDAE

1.	RM cross-vein absent. M not forked. Antenna of female with 12–14 segments	*Leptoconops* group (p. 14)
	RM cross-vein present. M forked. Antenna of female with 15 segments	2
2.	Empodium well developed, in female at least	*Forcipomyia* group (p. 14)
	Empodium small or rudimentary	3
3.	M petiolate, that is, forking at a point distal to level of cross-vein	4
	M sessile, that is, forking at level of cross-vein or proximal to this level	7
4.	R_2 usually present, two radial cells, either or both of which may be obliterated. Macrotrichia usually more abundant	5
	R_2 present or absent ; when present the second radial cell usually longer than the first. Macrotrichia scanty or entirely wanting	*Stilobezzia* group (p. 22)
5.	First radial cell completely or almost completely obliterated ; second, if open, oblong or square, characteristically square-ended. No distinct humeral pits. Claws of female small, equal. Antenna of male with last 4 segments elongate	*Dasyhelea* group (p. 17)
	Radial cells otherwise formed, both usually more or less open, second not with square end. Humeral pits distinct. Antenna of male with last 3 segments elongate	6
6.	Claws of female small, equal	*Culicoides* group (p. 18)
	Claws of female longer, equal or unequal	*Ceratopogon* group (p. 20)
7.	Thorax narrowed in front, and so more or less conical ...	*Macropeza* group (p. 24)
	Thorax not narrowed in front	8
8.	R_2 present, two radial cells	*Palpomyia* group (p. 25)
	R_2 wanting, a single radial cell, which is more or less open to the level of the cross-vein	*Bezzia* group (p. 28)

All these groups are not of equal value. The first, the *Leptoconops* group, is quite isolated from the other genera and should perhaps be separated off as a distinct subfamily—Leptoconopinae End., 1936. The others are all closely akin, and forms are common which in some respects connect them. I have therefore dispensed with the subfamily names Forcipomyiinae, Ceratopogoninae, Palpomyiinae and Bezziinae used by Enderlein (1936). All the genera included in the *Macropeza* group might have been placed either in the *Palpomyia* group or in the *Bezzia* group, but as this could have been done only by arbitrary means I have preferred to keep them apart. It would have been necessary, for example, to separate the two species of *Jenkinshelea* recorded from Africa.

Leptoconops Group

(Leptoconopinae Enderlein, 1936)

1. Lamellae very short. Frons with numerous spines or hairs *Styloconops* K., 1921
   Type : *albiventris* (de Meij.)
   (Syn. : *Acanthoconops* Carter, 1921)

   Lamellae elongate. Frons bare or with a single pair of hairs between the eyes 2

2. Antenna of female composed of 14 segments ... ... *Leptoconops* Skuse, 1890
   Type : *stygius* Skuse
   (Syns. : *Tersesthes* Towns., 1893 ; *Centrotypus* Grassi, 1901 ; *Mycterotypus* Noé, 1905 ; *Mycteromyia* Lutz, 1912, nec Phil. ; *Schizoconops* K., 1918 ; *Protersesthes* K., 1921)

   Antenna of female composed of 13 segments ... ... *Holoconops* K., 1918
   Type : *kerteszi* (K.)
   (Syns : *Leptoconops* auct. partim ; *Mycterotypus* auct. partim)

   Antenna of female composed of 12 segments ... ... *Microconops* K., 1921
   Type : *vexans* K.

The above is the classification suggested by Carter (1921) in his revision of the genus *Leptoconops*, with one addition, the genus *Microconops*, which had not been described at the time when Carter wrote. This classification has been adopted substantially by all recent writers on the group. It is remarkable for its simplicity and for the fact that it dispenses with the use of a number of the generic names recognized by Kieffer.

Forcipomyia Group

1. Microtrichia large and conspicuous, Macrotrichia when present scattered, suberect, not scale-like. Fringe on posterior border of wing simple, composed of a single row of alternating short and very short, simple, straight hairs. Costa reaching well beyond middle of wing. Second radial cell longer than first and usually open ... 2

   Microtrichia minute. Macrotrichia more abundant, covering the greater part of wing, sloping, often scale-like. Fringe more complex, not a single row of hairs. First radial cell narrow, often obliterated 3

2. Thorax without anterior tubercle. Costa extending about three-quarters length of wing. Basal segments (4–10) of antenna of female short, the first few flattened, subspherical, or at most not much longer than broad ... *Atrichopogon* K., 1906
Type : *exilis* (Coq.)
(Syns. : ? *Didymophleps* Weyen., 1883 ; *Kempia* K., 1913 ; *Gymnohelea* K., 1921 ; *Lophomyidium* Cordero, 1929 ; *Psilokempia* End., 1936)

Thorax with anterior tubercle. Costa extending more than four-fifths length of wing. Basal segments (4–10) of antenna of female more elongate, 1–5 times as long as broad ... ... *Dolichohelea* Edwards, 1929
Type : *polita* Edwards

3. Second radial cell longer than first, very narrow. Fringe on posterior border of wing composed of a row of long hairs between two rows of shorter, oblique hairs ... *Lasiohelea* K., 1921
Type : *stylifer* (Lutz)
(Syn. : *Centrorhynchus* Lutz, 1913)

Costa usually not reaching beyond middle of wing, or reaching only slightly beyond it. First radial cell narrow, often obliterated ; second not much longer, often triangular, sometimes obliterated. Fringe on posterior border of wing composed of hairs which are usually lanceolate, and sometimes pubescent or subplumose ... ... *Forcipomyia* subgroup (p. 16)

Atrichopogon. In this genus the eyes may be hairy all over, quite bare, or pubescent to some extent between these two extremes ; and the wings may bear macrotrichia all over the distal half and along the posterior border, or over only some part of this area, or they may be devoid of macrotrichia. Genera founded on these characters, but not differing in other essential respects from *Atrichopogon*, must therefore be regarded as synonyms. Such genera are *Kempia* K., 1913, and *Gymnohelea* K., 1921, founded for species with hairy eyes, and *Psilokempia* End., 1936, founded for *A. appendiculata* Goet., a species with the wings devoid of macrotrichia. *Lophomyidium uruguayense* Cordero appears to be a typical *Atrichopogon.*

Didymophleps. I have nothing further to add to the opinion expressed by Dr. Ingram and myself (1931) about this genus, namely, that, although it is just possible that it may be the same as *Atrichopogon* (in which case the name would have priority), ' Weyenbergh's description, and rather unconvincing figures, of *D. hortorum*, the type species, would, however, apply almost equally well to several other genera, in proof of which it will be sufficient to recall that Kieffer regarded *Didymophleps* as one of the *Bezzia* group of genera, and Johannsen (1905) has stated that it is " a synonym of either *Ceratopogon* or *Culicoides.*" Unless Weyenbergh's type of *D. hortorum* is still in existence and can be re-examined, the genus *Didymophleps* must inevitably remain for ever obscure.'

Lasiohelea. The name *Centrorhynchus* originally proposed for this genus by Lutz (1913) is preoccupied, having been used by Lühe (1911) for another genus (Vermes). Some of the species are known to bite mammals and to suck

blood. They are the only species of the group known to do this. In all the males in which the hypopygium has been examined in detail it is of a characteristic form which differs decidedly from that found in other genera of the group.

Forcipomyia Subgroup

1.	Antenna of female with last 6 segments elongate. Empodium large and broad, adapted for clinging. T.R. 3 or more. Parasitic on the wings of dragonflies	*Pterobosca* Macfie, 1932 Type: *aeschnosuga* (de Meij.)
	Antenna of female with last 5 segments elongate... ...	2
2.	Antenna of female with basal segments (4–10) short, the first few often broader than long, as in *Atrichopogon* ...	3
	Antenna of female with basal segments not so short, often vasiform or flask-shaped	4
3.	Male without empodium. Hypopygium characteristic, with harpes more or less H-shaped	*Apelma* K., 1919 Type : *auronitens* K.
		(Syns. : *Trichohelea* Goet., 1920 ; *Prosapelma* K., 1925)
	Male with empodium well developed, as in female. Hypopygium characteristic, with deep excavation in 9th sternite	*Thyridomyia* Saunders, 1925 Type : *palustris* Saunders
4.	Bearing scales in addition to bristles and hairs	*Lepidohelea* K., 1917 Type : *chrysolophus* (K.)
	Without scales	*Forcipomyia* Meg., 1818 Type : *bipunctata* (L.)
		(Syns. : *Helea* auct. ; *Ceratopogon* auct. ; *Labidomyia* Steph., 1829 ; *Tetraphora* Phil., 1865 ; *Prohelea* K., 1911 ; *Microhelea* K., 1917 ; *Phasmidohelea* Mayer, 1937)

A number of genera and subgenera have been described which must be regarded as synonyms of *Forcipomyia*. Those separated in the foregoing key are perhaps the most distinctive, but with the exception of *Pterobosca*, and possibly *Apelma*, they cannot be regarded as really distinct from *Forcipomyia*. The others are briefly referred to below.

Edwards (1926) has adopted a convenient means of grouping by the T.R. (tarsal ratio) in the case of the British species. He divides the species into four groups in which the T.R. is 0·4–0·65, 0·75–1·2, 1·35–1·75, and 2 or more respectively, and although, as he says, ' the dividing lines between the four groups may appear rather arbitrary on paper it will be found in practice that they are quite easy of application '. The form of the hypopygium of the males may furnish another, and more natural, means of grouping when it (and particularly the harpes or parameres) has been examined in detail in a sufficiently large number of species. Several distinct types of hypopygium may already be distinguished. There is, for example, a rather remarkable group of species in which the hypopygium is of the type found in *F. ingrami* Cart. or *F. titillans* (Winn.).

Euforcipomyia. This genus was founded by Malloch (1915) for those species of *Forcipomyia* in which the first tarsal segment of the hind legs is much longer than the second, i.e., with T.R. more than 1. In the type-species, *hirtipennis* Mall., the T.R. is about 1·5. Some of the insects which have been assigned to this genus are probably species of *Apelma*, *Lasiohelea* or *Thyridomyia.*

Microhelea. This genus was founded by Kieffer (1917) for *Atrichopogon microtomus* K. (not *tropicus*, as originally stated), a species resembling *Forcipomyia* but with the wings devoid of macrotrichia. As I have suggested elsewhere (1939), this insect may well have been a denuded specimen of *Forcipomyia inornatipennis.*

Phasmidohelea. Mayer (1937) founded this genus for a *Forcipomyia*-like species, *crudelis* Mayer, and associated with it two other species, *F. ixodoides* Feibrig-Gertz, 1928, and *F. obesa* da Costa Lima, 1928. All three species are parasitic on Phasmids. This fact alone would not justify the creation of a separate genus, and, as the other differential characters are, I think, of relatively small importance, I regard *Phasmidohelea* as a synonym of *Forcipomyia.*

Prohelea. This subgenus was founded by Kieffer (1911, 1912) for those species of *Forcipomyia* in which the first tarsal segment of the hind legs is shorter than the second, i.e., with T.R. less than 1. Some of the species assigned to it bear scales, others do not.

Prosapelma. Kieffer (1925) founded this genus for a European species, *cinerea* K., and referred to it two other European species, *meinerti* (K.) and *cilipes* (K.), which he had previously regarded as *Culicoides.* The genus has the characters of *Apelma*, but the two radial cells are distinct, the last antennal segment is without a stylet, and the claws are short. The empodium of *cinerea*, a female, is described as being almost wanting. Until further evidence is available I think that *Prosapelma* must be regarded as a synonym of either *Apelma* or *Culicoides.* Goetghebuer and Lenz, in their monograph on Palaearctic Ceratopogonidae (1933-34), referred *meinerti* and *cilipes* to the genus *Culicoides*; and Edwards (1939) included *meinerti* (of which he had examined a cotype) as a probable synonym of *Culicoides salinarius* K.

Tetraphora. What this genus really is will perhaps never now be known. Only a single species has been assigned to it, *fusca* Philippi, 1865. Edwards (1929) suggested that it might be an earlier name for *Dasyhelea*, but Ingram and Macfie (1931) thought it more likely to be a synonym of *Forcipomyia.*

Dasyhelea Group

1. Antennal segments sculptured; 12–14 in male binodose ... *Dasyhelea* K., 1911
   Type: *halophila* K.
   (Syns.: *Prokempia* K., 1913; *Pseudoculicoides* Mall., 1915; *Cryptoscena* End., 1936; *Dicryptoscena* End., 1936)

   Antennal segments not sculptured; 12–14 in male not binodose ... ... *Paradasyhelea* gen. nov.
   Type: *brevipalpis* (I. and M.)

General characters. Eyes hairy. Antennal segments usually sculptured. Antenna of male with last 4 segments elongate; 12–14 usually binodose. Humeral pits usually wanting. Wings unadorned. Microtrichia dense. Macrotrichia abundant, usually covering the greater part of the wing. Costa short, extending only to about middle of wing. Radial cells 2, small: first more or less obliterated; second also sometimes obliterated, but most commonly of a characteristic square-ended shape. M petiolate, usually forking only a short distance distal to cross-vein. Tarsi with 4th segment subcylindrical. T.R. usually more than 2. Claws of female all equal, short. Empodium quite rudimentary.

Both the distribution of the macrotrichia on the wings and the degree to which the radial cells may be open or obliterated vary to a considerable extent in different species, and often, too, in the two sexes of the same species. As pointed out by Edwards (1926), neither character is satisfactory as a basis for subdivision. For this reason the genera *Prokempia*, *Cryptoscena* and *Dicryptoscena* have not been adopted.

In a species described by Kieffer (1913) as *Culicoides insignicornis*, the antenna of the male is stated to have the last 4 segments elongate, as in *Dasyhelea*. Later (1925) Kieffer made the following brief reference to this insect: '*Culicoides insignicornis* Kieff. de l'Afrique tropicale est le type du genre *Tetrahelea* Kieff., à cause de ses antennes terminées par un stylet et munies de deux soies sensorielles aux articles deux-treize, mais surtout à cause des ailes sans soies microscopiques, en partie à soies longues, ayant D sessile et Cu soudé à R et grossi dans son tiers distal de façon à former une tache brune'. I have not been able to discover the description of the genus *Tetrahelea*, or any other reference to it. The description of *Culicoides insignicornis* suggests that it may be another synonym of *Dasyhelea*.

Culicoides Group

1. Antenna (in both sexes) with first segment large, about same size as torus ... ... *Diplosella* K., 1921
   Type: *sergenti* K.

   Antenna with first segment much reduced, smaller than torus 2

2. First segment of palp armed with a lateral tooth at its base ... *Oxyhelea* K., 1921
   Type: *dentatus* K.

   Palp unarmed ... ... *Culicoides* Latr., 1809
   Type: *pulicaris* (L.)

   (Syns.: *Oecacta* Poey, 1851; *Psychophaena* Philippi, 1865; *Haematomyidium* Goeldi, 1905; *Cotocripus* Brèthes, 1912; *Haemophoructus* Macfie, 1925; *Synhelea* K., 1925; ? *Prosapelma* K., 1925)

General characters. Eyes usually bare, but sometimes hairy. Antennal segments not sculptured. Antenna of male with last 3 segments elongate; 12–14 not binodose. Humeral pits generally conspicuous. Wings usually

adorned. Microtrichia dense. Macrotrichia usually abundant, but sometimes very scanty or absent. Costa comparatively short, usually extending more than half length of wing, but at most extending only to about level of end of Cu_1. Radial cells 2, small, usually subequal, the second not square-ended as in *Dasyhelea*; either or both may be obliterated, and sometimes they are confluent, R_2 being missing. M petiolate, forking at a point some distance distal to cross-vein. T.R. usually about 2. Tarsi with 4th segment short, sometimes cordiform. Claws of female all equal, small. Empodium small or rudimentary.

The genus *Culicoides* is now unwieldy. A small group of species, *C. stigma* (Mg.), etc., characterized by having the 4th tarsal segments cordiform, might be separated, but no real advantage would be served by doing this. It is probable that the characters of the hypopygium will eventually furnish a sound basis for a natural subdivision. At present, the characters of the hypopygium, and particularly those of the harpes or parameres, are known in only a comparatively small number of species, but already it is clear that they represent certain distinct groups.

Cotocripus. In a footnote to his key for the determination of the genera of Ceratopogonidae, Kieffer (1926) stated that he regarded *Cotocripus* as a synonym of *Dasyneura* (sic). The key was published after the death of its author, and the guess may be hazarded that *Dasyneura* is a misprint for *Dasyhelea.* I follow Edwards (1922), however, in regarding *Cotocripus* as a synonym of *Culicoides.*

Diplosella. Kieffer (1921, 1925) assigned four species of *Culicoides* to this subgenus, namely, *sergenti* K., *kribiensis* K., *biscapus* K. and *furens* Poey. Other authors who have examined specimens which they regarded as examples of the last species have not observed the peculiarity of the antennae characteristic of the genus *Diplosella*, and it is therefore possible that the specimens examined by Kieffer were abnormal, or that the appearance described by him may have been due to some unusual circumstance. The other species, so far as I am aware, have not been identified since Kieffer described them.

In all the genera of Ceratopogonidae the first segment of the antenna is present, but it is not always equally obvious, and in dried specimens especially is variable in appearance. The fact that Kieffer (1921) compared the appearance which he had observed in *C. sergenti* with that seen in the genera *Protersesthes* and *Centrorhynchus* may be regarded as evidence that it is not a character of generic importance.

Haemophoructus. The most characteristic feature of this genus is the single radial cell. Since it was described, the suppression of R_2 has been observed in several species of undoubted *Culicoides*, sometimes on only one wing while the other was normally formed. *Haemophoructus* must, therefore, I think, be regarded as a synonym of *Culicoides.*

Oecacta. I have followed the usual custom by regarding this genus as a synonym of *Culicoides.* The type-species, *furens* Poey, or what was believed to

be that species, has frequently been identified, and is a typical *Culicoides*. Kieffer (1921, 1925), however, considered that his genus *Diplosella* might be a synonym of *Oecacta*, if Poey had been in error (as seems possible) in stating that in the latter genus the wings are covered with scales.

Prosapelma. Kieffer (1925), who founded this genus for a European species, *cinerea* K., considered it to be akin to *Apelma*. He assigned to it, perhaps from memory only, two other species, *meinerti* K. and *cilipes* K., which he had previously regarded as *Culicoides*. Goetghebuer (1933) considers both the latter species to be *Culicoides*; and Edwards (1939), who has examined a cotype of *meinerti*, states that this species is undoubtedly a *Culicoides*, and perhaps a synonym of *salinarius* K. I hesitate to suppose that Kieffer could have described the macrotrichia of a *Culicoides* as resembling those of *Forcipomyia*, but the possibility must be borne in mind that *cinerea*, like *meinerti*, may really be a *Culicoides*, and the genus *Prosapelma* therefore a synonym of *Culicoides*.

Synhelea. This genus was erected by Kieffer (1925) for two species, *tropicalis* K. and *imicola* K., in which both the radial cells are obliterated. This character alone is probably not of great significance, and, as both species appear from their descriptions to be otherwise typical examples of *Culicoides*, I regard *Synhelea* as a synonym.

Ceratopogon Group

1. Claws of hind legs of female unequal. Wings adorned. Macrotrichia more or less abundant 2
   Claws of hind legs of female equal. Wings unadorned. Macrotrichia very scanty or entirely wanting 3
2. Second radial cell not formed. Antenna of male with terminal segments short... ... ... *Fanthamia* de Meillon, 1939
   Type : *adulator* de Meillon

   Second radial cell more or less clearly formed. Antenna of male with last 3 segments elongate ... ... *Alluaudomyia* K., 1913
   Type : *imparunguis* K.
   (Syns. : *Neoceratopogon* Mall., 1915 ; *Prionognathus* C., I. and M., 1921 ; *Thysanognathus* I. and M., 1922 ; *Isoecacta* Garrett, 1925)
3. M_2 entirely wanting. Costa not extending distally as far as level of end of Cu_1. Radial cells subequal, small or obliterated. No macrotrichia... ... ... *Brachypogon* K., 1899
   Type : *vitiosus* (Winn.)
   (Syn. : *Trishelea* K., 1925)

   M_2 not entirely wanting 4
4. M_2 complete or only narrowly interrupted at base. Costa extending distally as far as level of end of Cu_1. Radial cells subequal, longer than broad. Macrotrichia sometimes present ... ... *Ceratopogon* Mg., 1803
   Type : *communis* Mg.
   (Syn. : *Psilohelea* K., 1917)

   M_2 with about basal half wanting. Costa not extending distally as far as level of end of Cu_1. Radial cells subequal, small. No macrotrichia, or almost none ... *Isohelea* K., 1917
   Type : *lacteipennis* (Zett.)
   (Syn. : *Anakempia* K., 1924)

General characters. Eyes more or less hairy. Antennal segments not sculptured. Antenna of male with last 3 segments elongate (but ? *Fanthamia*) ; 12–14 not binodose. Humeral pits usually distinct. Microtrichia present or wanting. Macrotrichia usually absent, but sometimes present near tip. Costa short, extending at most to level of end of Cu_1. Radial cells 2, small or rather small, subequal ; either or both may be obliterated. M petiolate, forking some distance distal to level of cross-vein, but M_2 often partly, and sometimes completely, wanting. T.R. about 2. Tarsi with 4th segment short or cordiform. Claws of female rather large, equal or unequal. Empodium rudimentary.

The division of the species into groups according to the degree of development of M_2 as suggested by Edwards (1926), may be convenient, but is not likely to prove applicable when a larger number of species is known. One species has already been described, *C. natalensis* de Meillon, 1937, which will illustrate the difficulty. In it, M_2 is developed much as it is in *Ceratopogon*, but the radial cells are short and small, as they are in *Isohelea*.

Alluaudomyia. Some sixteen species have been referred to this genus or to one or other of its synonyms. Of them, one at least, *T. melanostictus* I. and M., 1923, is probably incorrectly placed, and should more suitably be regarded as a species of *Dasyhelea*. On the other hand, *Culicoides imperfectus* Goet., 1935, may really be a species of *Alluaudomyia* or *Fanthamia*. According to Johannsen (1934), *Isoecacta poeyi* Garrett, 1925, is a synonym of *A. bella* (Coq.).

Fanthamia. Two species have been referred to this genus, *adulator* de Meil. and *ornatipennis* de Meil., but the male of only one of them, the first, is known. As I have pointed out elsewhere (1939), the fact that the second radial cell is not formed is not without parallels, and alone this character would not be sufficient justification for separating the genus from *Alluaudomyia*. That, therefore, rests on the remarkable form of the antenna of the male *F. adulator*, which is apparently similar to that of the female. Intersex forms are sometimes met with among Ceratopogonidae in which a male body carries a female head, and it should be borne in mind that this male *F. adulator* may have been such a form. In this case there would be no reason to separate the genus *Fanthamia* from *Alluaudomyia*. The hypopygium of *F. adulator* is of a similar type to that of *Alluaudomyia*.

Nilohelea. This genus, described by Kieffer in 1921, should probably be included here. Only one species, *albipennis* K., has hitherto been described, and of it only the male is known. The wing is described as being ' sans poils, non ponctuée', the costa is short, not reaching to the middle of the wing, and both radial cells are obliterated. It is perhaps another synonym of *Isohelea*.

Stilobezzia Group

1. Wings without microtrichia. A single rather short radial cell, which is open to its base. Femora and tibiae all armed with spines. Tarsi with 4th segment not bilobed ... ... *Diaphanobezzia* I. and M., 1931
   Type : *pellucida* I. and M.

   Wings with microtrichia 2
2. Claws of hind legs of female very unequal, or a single long claw with or without a smaller basal barb ... 3

   Claws of female all equal 9
3. Tarsi with 4th segment bilobed. Claws of female unequal on all legs 4

   Tarsi with 4th segment cylindrical to bell-shaped, but not bilobed. Claws on four anterior legs of female all equal 6
4. Femora not armed with spines. Two radial cells, but first sometimes much reduced, and may even appear to be wanting ... ... *Stilobezzia* K., 1911
   Type : *notata* (de Meij.)=*festiva* K.
   (Syns. : *Hartomyia* Mall., 1915 ; *Neostilobezzia* Goet., 1934)

   Femora armed with spines 5
5. Two radial cells, both open. All femora armed with spines ... ... *Acanthohelea* K., 1917
   Type : *pruinosa* K.

   First radial cell obliterated, so only one radial cell visible. Only femora of fore legs armed... ... *Eukraiohelea* I. and M., 1921
   Type : *africana* I. and M.
6. First radial cell obliterated, so only one radial cell visible ... ... *Kiefferomyia* Mayer, 1937
   Type : *gorana* Mayer

   Two radial cells, both open 7
7. Femora of hind legs much swollen, and armed with numerous spines ... ... *Serromyia* Meg., 1818
   Type : *femorata* (Fabr.)
   (Syns. : *Prionomyia* Steph., 1829 ; *Ceratolophus* K., 1899 ; *Johannseniella* (Will.) K., 1910)

   Femora of hind legs not much swollen and without spines 8
8. Two radial cells separated for some distance by fusion of veins between them. Tarsi with 4th segment cordiform or bell-shaped... ... ... *Isthmohelea* I. and M., 1931
   Type : *disjuncta* I. and M.

   Radial cells not thus separated. Tarsi with 4th segment subcylindrical or cylindrical ... ... *Monohelea* K., 1917
   Type : *hieroglyphica* K.
   (Syns. : *Schizohelea* K., 1917 ; *Allohelea* K., 1917)
9. Only a single radial cell, R_2 wanting 10

   Two radial cells 11
10. Femora of fore legs armed with spines ... ... *Pseudobezzia* Mall., 1915
    Type : *expolita* (Coq.)
    (Syn. : *Allobezzia* K., 1917)

    Femora unarmed ... ... *Parabezzia* Mall., 1915
    Type : *petiolata* Mall.
11. Costa very long, extending to tip of wing where it is joined by R_{4+5}. Posterior end of abdomen not modified ... ... *Luciamyia* de Meillon, 1937
    Type : *biloba* de Meillon

    Costa shorter, not reaching to tip of wing, and not prolonged beyond its junction with R_{4+5}. Posterior end of abdomen peculiarly modified. Tarsi with 5th segments not inflated ... ... *Macrurohelea* I. and M., 1931
    Type : *caudata* I. and M.

Allohelea. Kieffer (1917) founded this genus for *Sphaeromias pulchripennis*, a species described by him in 1911. He included in it also two other species, *curriei* Coq. and *polita* Coq., notwithstanding the fact (which he mentions) that the form of the 4th tarsal segments in descriptions of these insects was not stated. The description of *pulchripennis* and the figures which illustrate it suggest that it is a *Monohelea*, and Dr. F. W. Edwards informs me that the type, which is in the collection of the British Museum, is in fact a *Monohelea.*

Diaphanobezzia. Only one species, *pellucida* I. and M., has been assigned to this peculiar genus, and of it only the male is known. Until females are discovered it is not possible to place the genus satisfactorily, so it is given here, first in this group, chiefly because in it the vein M forks some distance distal to the level of the cross-vein.

Luciamyia. The position of this genus is very uncertain. Only one species, *biloba* de Meil., has hitherto been described, and I should have supposed the single specimen of it studied—a female—to be an abnormal individual. I should like to suggest that this female may belong to the same species as the single male (taken in the same locality, and on the same day) described by de Meillon under the name *Monohelea irrita.* As pointed out by de Meillon himself, *M. irrita* does not really resemble a *Monohelea*, and cannot be admitted to that genus. It does not conform to the type of any other genus known to me.

Pseudobezzia and *Parabezzia.* I have not had the opportunity of examining specimens of the type-species of either of these genera, and I am in some doubt about them, because the descriptions given lack some details. Malloch states that, apart from the fact that M is petiolate, they correspond with *Bezzia* and *Probezzia* respectively, and I have assumed that in this he was correct.

Serromyia. In most of the species assigned to this genus the wing-vein M is shortly petiolate, but in others it is said to be just sessile, that is, forking at the level of the cross-vein. The form of the 4th tarsal segment is cordiform in some, cylindrical or subcylindrical in others. There are, too, notable differences in the characters of the claws, for whereas in most of the species those on the hind legs of the female are unequal, as in *Stilobezzia*, in others they are small and equal. All the species may not be congeneric. The characters here assumed to be generic are those of the type-species, *femorata* (Fabr.).

Stilobezzia. In this genus the two radial cells are usually well developed and both open, the second longer than the first. The first may, however, be much reduced in size and more or less obliterated; indeed, in some species it appears to be completely or almost completely absent. Such a species is *poikiloptera* I. and M., which was originally assigned to the genus *Parabezzia.* The hypopygium is similar to that found in other species of *Stilobezzia.*

Goetghebuer (1934) has proposed a division of the species of this genus into two sets, the one (*Neostilobezzia*) with, and the other (*Stilobezzia* s. str.) without, macrotrichia on the wings. I have not adopted this mode of division because of the sexual differences in this character met with in some species.

Macropeza Group*

1.	Wings very broad, especially at base, anal lobe large	*Jenkinshelea* Macfie, 1934 Type : *setosipennis* (K.)
	Wings long and narrow	2
2.	No microtrichia	*Pellucidomyia* Macfie, 1939 Type : *ugandae* Macfie
	Microtrichia present	3
3.	Cross-vein short or very short, M and R therefore close together, the cell between them at its widest part narrower than distance at same level between M and Cu or Cu_1. Costa not reaching tip of wing	4
	Cross-vein long, M and R therefore widely separated, the cell between them at its widest part not narrower than distance at same level between M and Cu or Cu_1. Tip of wing usually pointed. Costa usually reaching tip	5
4.	Tarsi of four anterior legs with 4th segment ending in two bifid lobes, each armed with a spine ; those of hind legs cylindrical, long. Claws of female unequal, simple, as in *Stilobezzia*. Femora of fore legs armed	*Tetrabezzia* K., 1917 Type : *spinigera* (K.)
	Tarsi with 4th segments subcylindrical to almost cordiform, not with two bifid lobes. Claws of female all equal, each with a basal barb. Femora unarmed	*Paryphoconus* Enderlein, 1912 Type : *angustipennis* Enderlein
5.	Costa extending for some distance beyond its junction with R_{4+5} ; radial cell very narrow ...	5
	Costa extending only a short distance beyond its junction with R_{4+5} ; at least distal part of radial cell wide or moderately wide	6
6.	Thorax sharply pointed in front, projecting well over head. Femora of fore legs thickened at apex. Cross-vein about middle of wing. Anal lobe very poorly developed, the angle very obtuse	*Calyptopogon* K., 1910 Type : *albitarsis* K.
	Thorax bluntly conical in front. Femora of fore legs slender or only slightly thickened at apex. Anal lobe well or moderately well developed, the angle rectangular or obtuse	*Macropeza* Meigen, 1818 Type : *albitarsis* Meigen
7.	Cell between costa and R widest at its distal end (♀). Tip of wing usually pointed. Cross-vein usually distal to middle of wing. A single radial cell ...	*Macroptilum* Becker, 1903 Type : *nudum* Becker
	Cell between costa and R very narrow at its distal end. Cross-vein about middle of wing. Two radial cells	*Macroptilum calcipennis* M., 1939

Tetrabezzia. So far as I am aware, only two species have hitherto been referred to this genus, *spinigera* (K.), an Indian species which was originally assigned to the genus *Dibezzia*, and *argentea* I. and M., a species found in

*See Macfie (1939).

West Africa. Only females are known. Notwithstanding its affinities with *Macropeza* (and *Paryphoconus*), the genus was separated off by Kieffer (1926) in his key. The reason for this separation is not very clear because one of the distinctions made concerns the antenna of the male, and, as noted above, no male *Tetrabezzia* is known. The unique female, the type of *spinigera*, is in the collection of the Indian Museum, Calcutta. The Director has very kindly examined it for me, and tells me that it is now much damaged, lacking both the hind legs and the greater part of both the middle legs, but that ' the thorax is certainly narrowed in front and so more or less conical.' Dr. F. W. Edwards has kindly re-examined the two specimens of *argentea*, which are in the collection of the British Museum. Both are mounted on slides, and it is difficult now to be sure of the shape of the thorax, but he reports that it seems to be ' somewhat pointed in front, and conical, certainly more so than in *Palpomyia*, though perhaps less so than in *Macropeza*.'

Dr. Edwards tells me, too, that there is in the collection of the British Museum a female from Borneo of a species which is evidently very close to the genotype of *Tetrabezzia*. In it, however, the thorax is broad in front, not appreciably narrowed as seen from above. Perhaps, then, there may be two separate genera confused here, the one (e.g., *spinigera*) belonging to the *Macropeza* group, and the other (e.g., the Borneo species) to the *Bezzia* group. The genus will therefore be placed a second time in the key to genera of the *Bezzia* group.

It may also be pointed out here that, apart from the fact that there are two radial cells, the genus *Metahelea* resembles *Tetrabezzia* in many respects.

Palpomyia GROUP

1.	Tarsi with 4th segment ending in two bifid lobes armed with spines; 5th segment unarmed. Femora armed. Claws of female on at least four posterior legs* unequal, simple (or a single claw with a basal barb), i.e., as in *Stilobezzia*... ...	*Metahelea* Edwards, 1929 Type : *metallescens* Edwards
	Tarsi with 4th segment subcylindrical, cordiform, or bilobed, but not with two bifid lobes	2
2.	Claws of hind legs of female unequal, simple (or a single claw with a basal barb), i.e., as in *Stilobezzia*	3
	Claws of hind legs of female equal and simple or barbed, or if unequal then not simple but each with a basal barb	5
3.	Tarsi with 5th segment inflated on fore legs, unarmed on all legs; 4th segment on four posterior legs deeply bilobed, armed with spines. Femora not armed	*Clinohelea* K., 1917 Type : *unimaculata* (Mcq.)
	Tarsi with 5th segment on fore legs not inflated. Femora armed	4

*The tarsi of the fore legs are missing from the unique specimen of *Metahelea metallescens* Edwards.

4. Femora of fore legs greatly swollen. Tarsi with 5th segment not armed. Claws equal and barbed on four anterior legs, a single long claw with a basal barb on hind legs ... ... *Heteromyia* Say, 1825
Type : *fasciata* Say
(Syn. : *Pachyleptus* Walker, 1856)

Femora of fore legs not much swollen. Tarsi with 5th segment armed with batonnets. Claws unequal and simple on all legs ... ... *Xenohelea* K., 1917
Type : *pruinosa* K.
(Syn. : *Mixohelea* K., 1917)

5. Claws of female not all similar : equal and barbed on fore legs, unequal and barbed on four posterior legs. Tarsi with 4th segment subcylindrical, not cordiform ; 5th segment armed with batonnets. Femora armed or unarmed ... *Dicrohelea* K., 1917
Type : *filicornis* (K.)

Claws of female all equal or unequal, but if unequal not always unequal to the same degree 6

6. Claws of female all unequal and barbed, but more unequal on hind legs than on others. Tarsi with 4th segment subcylindrical ; 5th segment armed. Femora unarmed. Abdomen petiolate... ... *Dibezzia* K., 1911
Type : *clavata* K.

Claws of female all equal and simple. Tarsi with 4th segment cordiform or nearly so ; 5th segment armed. Femora usually armed ... ... *Homohelea* K., 1917
Type : *abjuncta* (K.)

Claws of female all equal and barbed 7

7. Costa prolonged beyond R_{4+5}. Tarsi with 4th segment cordiform ; 5th segment unarmed, inflated on fore legs ... ... *Neurohelea* K., 1925
Type : *luteitarsis* (Mg.)

Costa not prolonged beyond R_{4+5}. Tarsi with 5th segment not inflated 8

8. Femora armed with spines 9

Femora unarmed 10

9. Tarsi with 4th segment subcylindrical ; 5th segment armed with batonnets ... ... *Sphaeromias* Curtis, 1829
Type : *fasciatus* (Mg.)
(Syns. : *Xylocrypta* K., 1899 ; *Schizodactylus* I. and M., 1921 ; *Ankistrodactylus* I. and M., 1922)

Tarsi with 4th segment cordiform ; 5th segment not armed with batonnets... ... ... *Palpomyia* Mg., 1818
Type : *flavipes* (Mg.)
(Syns. : *Apogon* Rond., 1856 ; *Pachyleptus* Walk., 1856 ; *Alasion* Rond., 1857)

10. Tarsi with 5th segment armed with batonnets ... *Johannsenomyia* Malloch, 1915
Type : ? *bimaculata* (Loew)
(Syns. : *Johannseniella* auct. partim ; *Sphaeromias* Kieff. nec Curt.)

Tarsi with 5th segment unarmed ... ... *Diplohelea* K., 1925
Type : *parvula* K.

Dibezzia. In his key to the genera of Ceratopogonidae, Kieffer (1926) included this genus in the *Bezzia* group. This must, I think, have been a mistake, because, although he later referred divergent forms to it, the type-species and the two others described with it all have two radial cells. The claws of one species, *brevistila*, are not described; those of the other two are dissimilar. The claws of *clavata* are described (and figured) as being unequal and barbed on all the legs. Those of *longistila* are described as being equal and barbed on the fore legs, unequal and barbed on the four posterior legs—that is, as they are in the genus *Dicrohelea*—and therefore, I believe, this species should be considered to belong to the latter genus, not to *Dibezzia*.

Dicrohelea. In the original description (and figure) of *Palpomyia filicornis*, the type-species of this genus, Kieffer (1910) showed the claws of the anterior legs as unequal, the one long and bifid, the other short and barbed, not subequal and barbed as they are in all the other species which he assigned to the genus. This was presumably only an error in the interpretation of the appearances which Kieffer intended thus tacitly to correct.

Heteromyia. Dissimilar forms have been assigned to this genus, some of which should probably be regarded as species of *Palpomyia*. This has led some authors to include the genus in *Palpomyia*. Further confusion has been caused by doubts about the venation of the wing, which, as pointed out by Johannsen, is incorrectly shown in Say's figure; and by the fact that in differentiating the genus Kieffer laid more stress on the form of the femora and tibiae of the fore legs than he did on the characters of the tarsal segments and claws.

I am indebted to Dr. F. W. Edwards for the following brief summary of the chief characters of a female specimen of *H. fasciata* Say, the type-species, which is in the collection of the British Museum. Thorax broad, with anterior tubercle. Wings as in *Palpomyia*. Four anterior legs with 4th tarsal segment bilobed, unarmed; 5th unarmed; claws equal, barbed. Hind legs with tarsi very long: 4th segment cylindrical; 5th unarmed; a single, very long claw, barbed at base. Tibia of fore leg curved to fit the swollen femur, and with a stout, blunt, thumb-like projection at tip.

Neurohelea and *Diplohelea.* In these two genera the wing-vein M is not clearly sessile, but forks at, or almost at, the level of the cross-vein, and may be very shortly petiolate. They appear, however, to be more nearly related to *Palpomyia* than they are to *Stilobezzia*, and they are therefore included in this group.

Pachyleptus. The characters of this genus, as given by Walker, agree with those of *Heteromyia*, and the two may be regarded as synonymous. The venation of the wing in *Pachyleptus* is not described in detail by Walker, and the veins are merely stated to be 'like those of *Ceratopogon*.' According to Say, there is only a single radial cell in *Heteromyia*, but Johannsen, who re-examined the type, states that this is an error, and that there are in fact two radial cells. It must therefore be assumed that in *Heteromyia*, and so presumably in

Pachyleptus, there are normally two radial cells. In Arribalzaga's (1894) species of *Pachyleptus*, *P. antequerae*, there is only a single radial cell. If it was in fact a *Pachyleptus*, it must then have been an unusual form, in which R_2 was absent. The species described by Kieffer (1911) from the Seychelles, *P. rufipes* (♀), in which also there is only a single radial cell, is different, for the claws are small and equal on all the legs. It is presumably either a *Bezzia* or a *Palpomyia* in which, as sometimes happens, R_2 is lacking.

Xenohelea and *Mixohelea*. These two genera were both described by Kieffer (1917) in the same paper, the one in the synoptic table at its beginning, and the other in a supplement at its end. In the one, *Xenohelea*, the 4th tarsal segments are said to be cylindrical, in the other, *Mixohelea*, they are cordiform; but it is not easy to uphold this distinction, because intermediate forms occur. In *X. pruinosa*, the type-species of the first genus, which was more fully described in the following year (1918), the 4th tarsal segments are said to be ' a little longer than broad, not cordiform '; in the five species cited by Kieffer as belonging to the genus *Mixohelea*, they are variously described as ' cordiform but not bilobed', 'hardly longer than broad', and 'a little longer than broad'. In view of these facts I think that the genus *Mixohelea* must be considered as a synonym of *Xenohelea*.

Bezzia Group

RM cross-vein very short, the cell between R and M therefore very narrow or even obliterated. Costa long, extending to tip of wing. M sessile : in male forking at level of cross-vein ; in female forking well proximal to this level, and with M_2 at first strongly curved towards base. Tarsi with 4th segment spoon-shaped or subcylindrical, not bilobed ; 5th unarmed. Femora unarmed. Claws small, equal ... *Stenoxenus* Coquillet, 1899
Type : *johnsoni* Coquillet

RM cross-vein longer, the cell between R and M well formed and not very narrow. M sessile, forking at level of cross-vein or more proximally, but M_2 not at first curved towards base .. .. *Bezzia* subgroup (p. 29)

Stenoxenus. Coquillet (1899) considered this genus to belong to a separate family, Stenoxenidae, but Kieffer (1906) placed it in a subfamily, Stenoxeninae, of Chironomidae, and later (1909) pointed out its affinities with the genus *Bezzia* and referred it to Ceratopogonidae. The unusual form of M_2 which is perhaps the most obvious characteristic of the genus, is not found in the only male known, that of *S. dimorphus* K. In the *Bezzia* group of genera it most closely resembles *Dicrobezzia*. In that genus also the costa is prolonged, but the 4th tarsal segment is cordiform, as in *Bezzia*, and the 5th, in the female, is armed with batonnets. In some respects it bears a superficial resemblance also to *Macroptilum*, but differs notably in two respects, namely, in having the thorax broad anteriorly, not narrowed and conical, and in having the cross-vein very short instead of long.

Bezzia Subgroup

1. Tarsi of four anterior legs with 4th segment ending in two bifid lobes, each armed with a spine ; those of hind legs cylindrical, long. Claws of female unequal, simple, as in *Stilobezzia*. Femora of fore legs armed. Hind legs very long ... ... *Tetrabezzia* K., 1917
   Type : *spinigera* (K.)

   All tarsi with 4th segment bilobed or cordate 2
   All tarsi with 4th segment subcylindrical 5
2. Tarsi of fore legs with 5th segment inflated. Claws of female equal on fore legs, unequal on four posterior legs. Femora not armed. Thorax with an erect spine... ... *Ceratobezzia* K., 1917
   Type : *fallax* K.

   Tarsi of fore legs with 5th segment not inflated. Claws of female equal on all legs 3
3. Costa in female very long, reaching nearly to tip of wing. Tarsi with 5th segment armed. Femora unarmed ... *Dicrobezzia* K., 1919
   Type : *venusta* (Mg.)

   Costa in female not so long, and 5th tarsal segments not armed 4
4 Femora of at least fore legs armed with spines ... ... *Bezzia* K., 1899
   Type : *ornata* (Mg.)
   (Syn. : *Lasiobezzia* K., 1925)

   All femora unarmed ... ... *Probezzia* K., 1906
   Type : ? *albiventris* (H. Loew)
5. Femora of fore legs armed with stout spines ; others unarmed. Tarsi with 5th segment unarmed. Claws of female equal, barbed ... ... *Homobezzia* Macfie, 1932
   Type : *nyasae* Macfie

   All femora without stout spines (*dents*), but those of four posterior legs armed with longer spines (*spinules*). Tarsi with 5th segment armed. Claws of female equal, barbed *Nilobezzia* K., 1921
   Type : *armata* K.

   Femora not thus armed 6
6. Tarsi with 5th segment armed. Claws of female equal, barbed ... ... *Parrotia* K., 1924
   Type : *flaviventris* K.

   Tarsi with 5th segment unarmed. Palpi very short. Antenna of male with segments 3–12 bearing three or four whorls of hairs ... ... *Crespinia* K., 1924
   Type : *brevipalpis* K

Bezzia and *Probezzia*. The general consensus of opinion seems to be that the separation of these two genera is unnatural because it rests on but a single, rather variable character.

Crespinia. Only the male of but a single species of this genus is known. It is said to differ from *Probezzia* in having the 4th tarsal segment not cordiform, in the form of the palps, and in the number of whorls of the antennal plume. Until females are discovered the status of the genus must remain uncertain.

Lasiobezzia. This genus, described by Kieffer (1925) in a note of less than four lines, was founded to receive a single species, *Bezzia pilipennis* Lundstr., 1916, which differs from all other species of *Bezzia* in having macrotrichia on the wings. Kieffer does not seem to have himself seen any specimen of the

species, and it has not again been identified. It may well prove that the macrotrichia were merely adventitious. The genus is therefore regarded as a synonym of *Bezzia* until further evidence of its validity is forthcoming.

Parrotia. This genus differs from *Nilobezzia* in, apparently, only one respect, namely, in having no long spines (*spinules*) on the femora. These *spinules* of *Nilobezzia* are spine-like bristles which do not differ greatly from ordinary bristles, and intermediate forms may be found, about which it is not easy to be sure whether they are long spines or strong bristles. As I have suggested elsewhere (1934), they may prove to be unreliable as a character for generic differentiation.

Tetrabezzia. See note on *Macropeza* group (p. 24).

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CULTURE OF *TRYPANOSOMA GAMBIENSE* IN BLOOD FROM NORMAL AND INFECTED PERSONS

BY

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This note describes preliminary experiments on the culture of *T. gambiense*. During a recent visit to Leopoldville, Dr. P. Brutsaert and Dr. C. Henrard kindly demonstrated their technique for the cultivation of trypanosomes upon a medium containing human blood (Brutsaert and Henrard, 1938), and mentioned that they had found that the blood of some individuals was more suitable than that of others. In the present work an attempt has been made to investigate whether these differences could be associated with previous exposure to infection by trypanosomes. Owing to limitations of time, connected with the outbreak of war, it was not possible to do more than one experiment.

TECHNIQUE

The technique was that described by Brutsaert and Henrard (1938), the medium consisting of 2 ml. each of Ringer solution† and of blood containing 1 per cent. sodium citrate. For inoculations, 5 ml. blood was withdrawn from the infected animal or man and mixed with 1 ml. of a 1 per cent. solution of 'liquoïde Roche' (sodium polyanethol sulphonate). This prevents clotting; apparently the use of citrate at this stage is injurious to the trypanosomes. About 0·2 ml. of the infected blood was added to each tube, which was then kept at room-temperature (28–30° C.). Examination of the cultures was made at intervals between the 10th and 20th days, and all tubes in which living parasites were found were considered as positive. In most cases the forms present were of the long and slender type, and they tended to be grouped together in small rosettes.

STRAINS OF TRYPANOSOMES

1. Saidi Zendewa, a newly diagnosed case of sleeping sickness; scanty trypanosomes were seen in stained films of blood.

2. Yeye, an arsenic-resistant strain obtained from the Belgian Congo, through the kindness of Dr. L. Van Hoof; it was fairly virulent for guinea-pigs, in which it had passed through 6 passages during 8 months. Blood from a guinea-pig containing many trypanosomes was inoculated into several tubes, and one of these which developed a vigorous culture was used to inoculate the present series.

3. Monkey 61, a chronic strain isolated a few months previously and maintained in monkeys at the Gadau Laboratory; few trypanosomes were present in the blood.

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† NaCl, 0·65 gm.; KCl, 0·014 gm.; $CaCl_2$, 0·012 gm.; H_2O, 100 ml.

4. Das, a monkey which had been inoculated by Dr. J. L. McLetchie from a human case in the Das region of Nigeria ; the infection was very chronic and few trypanosomes were present in the blood.

5. Musa Gonka, a newly diagnosed case of sleeping sickness ; trypanosomes were seen in gland-juice but none in thick films of the blood stained with Giemsa.

SOURCE OF SPECIMENS OF BLOOD

These were arranged in two groups :

I. Persons who had not been appreciably exposed to infection.

II. Patients who had been treated for sleeping sickness, and persons frequently exposed to the bites of tsetse-flies.

GROUP I

A : a European (F. H.).

B, C and F : African laboratory assistants.

D and E : African domestic servants.

GROUP II

G : Saidu Madare, an old sleeping sickness case who had received much treatment ; last injection received 5 months ago.

H : Ali Shira, similar ; last injection received 3 weeks ago.

I : Buba Sokoto, a patient suspected of sleeping sickness, but no trypanosomes could be discovered in blood or gland-juice.

J : Garuba Gwonka, a patient with sleeping sickness. He had received the 2nd injection of a second course of antrypol (Bayer 205) treatment on the day before his blood was obtained ; 4 days later his plasma still contained 4·2 mgm. per 100 ml.

K : Saidi Zendewa, the new untested case, who provided strain 1 above. In the single case (strain 2) in which his blood showed a positive culture, it is possible that the trypanosomes were really derived from his own infection.

L : Umuru Azare, a fly-boy, exposed to tsetse-flies almost every day during his rounds in the bush ; three weeks earlier he had received an injection of tryparsamide in connection with other experiments.

RESULTS

The cases in which successful cultures were obtained are shown in the table. Some tubes developed bacterial contaminations ; these are shown crossed off in the table, and they have been left out of calculation. It is seen that the results are irregular. All strains were capable of cultivation, and all the bloods (except E) permitted growth in one or more cases. On the other hand, no strain developed in all the tubes, and no blood, except I, sustained growth of all the strains. The distribution of successful cultures seems to be fortuitous ; certainly trypanosomes grow as readily, if not more readily, in blood from the group exposed to infection as they do in the group of normal controls. Thus there was no evidence by this method that immunity had been acquired against a second infection. However, it must be noted that a period of two days elapsed between withdrawal of the blood and inoculation of the tubes, the medium being stored at room-temperature (28–30° C.), and there are suggestions that during this period labile trypanocidal constituents may have disintegrated. According to Laveran, and Adams, cited in the review by Culbertson (1935), the trypanocidal

TABLE

Showing the number of successful cultures obtained with various strains of *T. gambiense*, using blood from various individuals

Source of blood	Strain of trypanosomes					Percentage of positive cultures
	1	2	3	4	5	
A	+	+	O	+	+	80
B	+	+	+	O		75
C	O	O		O	+	25
D	O	O	+	+	O	40
E	O	O	O	O	O	0
F	O	+	+	O	O	40
G	O	O	+	+	O	40
H	+	O	+	O	O	40
I	+	+	+	+	+	100
J	+	+	+	+	O	80
K		+	O	O	O	25
L		+	O		+	66
Percentage of positive cultures	50	60	64	45	36	

power which normal human serum exerts on *T. brucei* is gradually lost in 2–12 weeks on storage at room-temperature or in the ice-box; and Reichenow (1934) emphasizes that it is advantageous to keep the medium 2–3 days in the ice-chest before use. Moreover, Reichenow found that a strain of *T. congolense* could be cultured with ease in a medium containing human blood, although human serum, which had stood 48 hours in the ice-chest, exerted a definite therapeutic action in rats infected with this parasite. Hence, caution is required in the interpretation of these results.

The growth of 80 per cent. of the strains in blood J, which contained considerable quantities of antrypol, is remarkable; it is in accordance with the view, based on the work of Jancsó and others, that this compound acts, not directly,

but in some indirect manner (possibly by an opsonic action in association with the phagocytes of the reticulo-endothelial system).

The cultures obtained with strain 5, in which no trypanosomes had been seen in thick films of the blood used for inoculation, support the contention by Brutsaert that trypanosomes can be disclosed by this technique even when direct examination of the blood has been unsuccessful.

SUMMARY

Cultures of five strains of *T. gambiense* were obtained by the technique of Brutsaert and Henrard, using a mixture of human blood and Ringer's solution at room-temperature. Trypanosomes grew as readily in blood from cases of sleeping sickness as they did in that from normal controls ; hence there was no evidence by this technique of immunity against a second infection. In one case trypanosomes grew readily in blood containing appreciable quantities of antrypol (Bayer 205), and in another case a culture was obtained from a patient whose blood showed no trypanosomes during examination of stained thick films.

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SOME OBSERVATIONS ON THE FLIGHT OF STAINED ANOPHELINES AT N'KANA, NORTHERN RHODESIA

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(*Malaria Control Officer*)

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Anopheline mosquitoes are usually regarded as weak fliers in comparison with other members of the order Diptera. Thus Patton (1905) demonstrated a flight of half a mile for *A. gambiae* in Arabia, and in 1909 Deaderick stated that half a mile could be regarded as the maximum limit of flight. This idea consequently set the limit of malaria control to a radius of half a mile, a distance which was generally thought to be sufficient even so late as 1933 by Watson, who, having in 1929 instituted half-mile control round a copper mine in Northern Rhodesia, wrote : ' There are possibly places where half a mile is not sufficient protection, but these are exceptional.' Actually the mine of which he was writing (the Roan Antelope) has itself proved an exception, for control has already had to be extended from half a mile to $1\frac{1}{2}$–2 miles on the side of the prevailing winds.

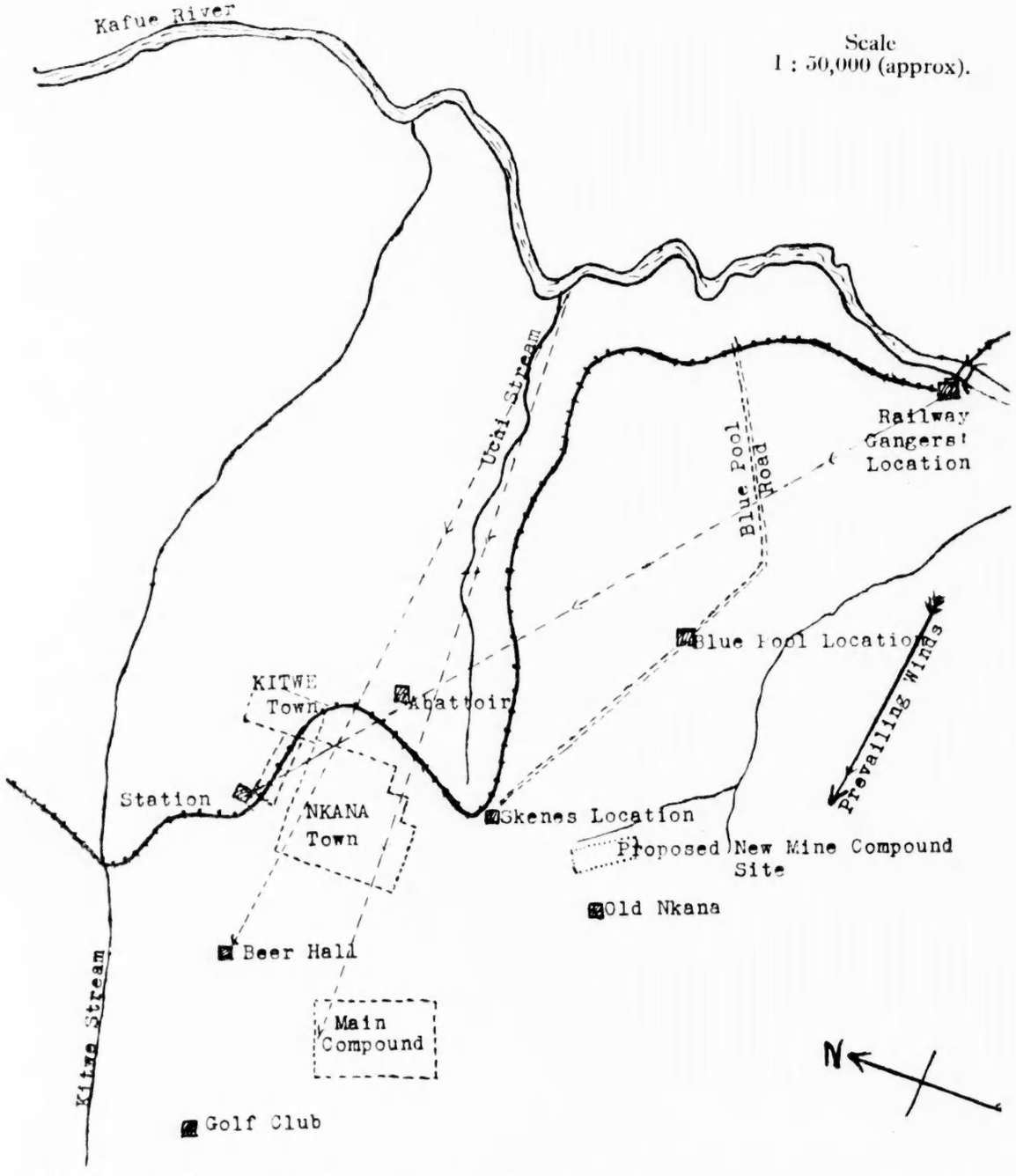
The detection in 1913 of long flights at Gatun, Panama, was probably the first indication that greater distances might be covered : adult anophelines were stained and liberated, and some were eventually recovered 6,250 ft. from the liberating station. Since that date, Le Prince and Griffitts (1917) and Geiger, Purdy and Tarbett (1919) have, by the liberation of stained anophelines, recorded flights of various distances up to 1·7 miles. According to Boyd (1930), however, the long-distance record appears to be that established by Van Breeman, who in 1918 recaptured in Batavia stained *A. ludlowii* as far as 6,200 metres from the point of liberation.

Concerning the range of flight of the principal malaria vectors in South Africa, i.e., *A. gambiae* and *A. funestus*, Swellengrebel, Annecke and De Meillon (1931) make the following remarks : ' No exact data on this point are available in the Union ' ; and again : ' In South Africa the validity of the half-mile radius, as a basis to fix the boundary of the area of anti-larval operations, still requires further confirmation.' Since these statements were made, it has been my privilege to assist one of the authors, Dr. B. De Meillon, in flight-experiments at N'Kana, Northern Rhodesia, where distances up to 2·80 miles were recorded down-wind in 1937.

In 1939, with the assistance of my colleague, Mr. K. Campbell, I made further flight-tests at N'Kana, with the results here recorded.

N'Kana is a copper-mining township on the Northern Rhodesia-Congo border, about 170 miles north of Broken Hill. The European population numbers approximately 2,200, while Kitwe, the adjoining commercial town situated half

a mile north-east, has a population of about 300. The malaria problem in these towns has, I think (as I shall endeavour to prove), been greatly influenced by the Kafue River three miles eastward. A permanent and abnormally heavy focus of *A. funestus* breeding-places is situated along the banks of this river, to windward of the towns. The winds prevail from east to south (see sketch-map), i.e., from the Kafue River directly across N'Kana and Kitwe. It was to prove,



Sketch-map of N'Kana, Northern Rhodesia, showing maximum flights only of *Anopheles funestus* and *Anopheles gambiae* (greatest distance covered, 4·50 miles).

if possible, flight down-wind from the Kafue to N'Kana that our more recent tests were made.

Malaria control at N'Kana began in 1929, with anti-larval measures carried out over the usual radius of half a mile from the edge of the inhabited areas. Gradually this was increased on the windward side to one and a half miles; but, despite efficient control, anopheline imagines were constantly to be found in the native huts (in the control area) to windward.

In April, 1937, Dr. De Meillon was called in to investigate the position. Tests were carried out, and flights of 2·80 miles down-wind were recorded with both *A. gambiae* and *A. funestus*. Dr. De Meillon recommended drainage to be extended eastwards along the Uchi dambo (marsh) to the Kafue. This was done, and the drains were oiled weekly; yet still the catches of imagines in N'Kana remained fairly constant. It therefore seemed obvious that at least a certain percentage of the imagines must be migrating from the Kafue River, which is three miles away at its nearest point.

Accordingly I reported in March, 1939:

'The Kafue River as a breeding-ground for *A. funestus* is amply demonstrated by the following facts:

'(*a*) *A. funestus* larvae were found in the slow-moving water along the side of the west bank.

'(*b*) Adult *A. funestus* captured in four native huts near the pumping-station on the Kafue bank totalled 391 in one day.

'(*c*) Adult *funestus* were taken in a railway culvert near the railway bridge that crosses the Kafue.'

Flight-experiments were then undertaken, with the following results:

April 2nd, 1939; 6 p.m. (sunset); winds south-east, Beaufort scale no. 3, approximately 8-12 miles per hour.

500 (450)* *A. funestus* and *A. gambiae* released at the confluence of the Uchi stream and the Kafue River; stained with 2 per cent. aqueous solution of eosin.

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*Experiments which we have carried out indicate that some 10 per cent. of stained mosquitoes lose their staining during an observation-period. Professor R. M. Gordon, of the Liverpool School of Tropical Medicine, has kindly informed me that 'out of a total of 32 control mosquitoes retained out of some 150 stained, 100 per cent. were found positive over a period of up to 8 days after staining.' Personally I have been less fortunate: three tests made in June to ascertain the percentage stained gave the following results:

Test no.	Anophelines caged	No. positive for stain after 2 days	Percentage positive
1	1,080	970	89
2	55	48	86
3	160	150	94
		Approximate average	90

Here and elsewhere in this paper, therefore, after the figure representing the number of anophelines released, a second figure is given in parentheses to show the probable number of mosquitoes which were actually stained at the time of release.

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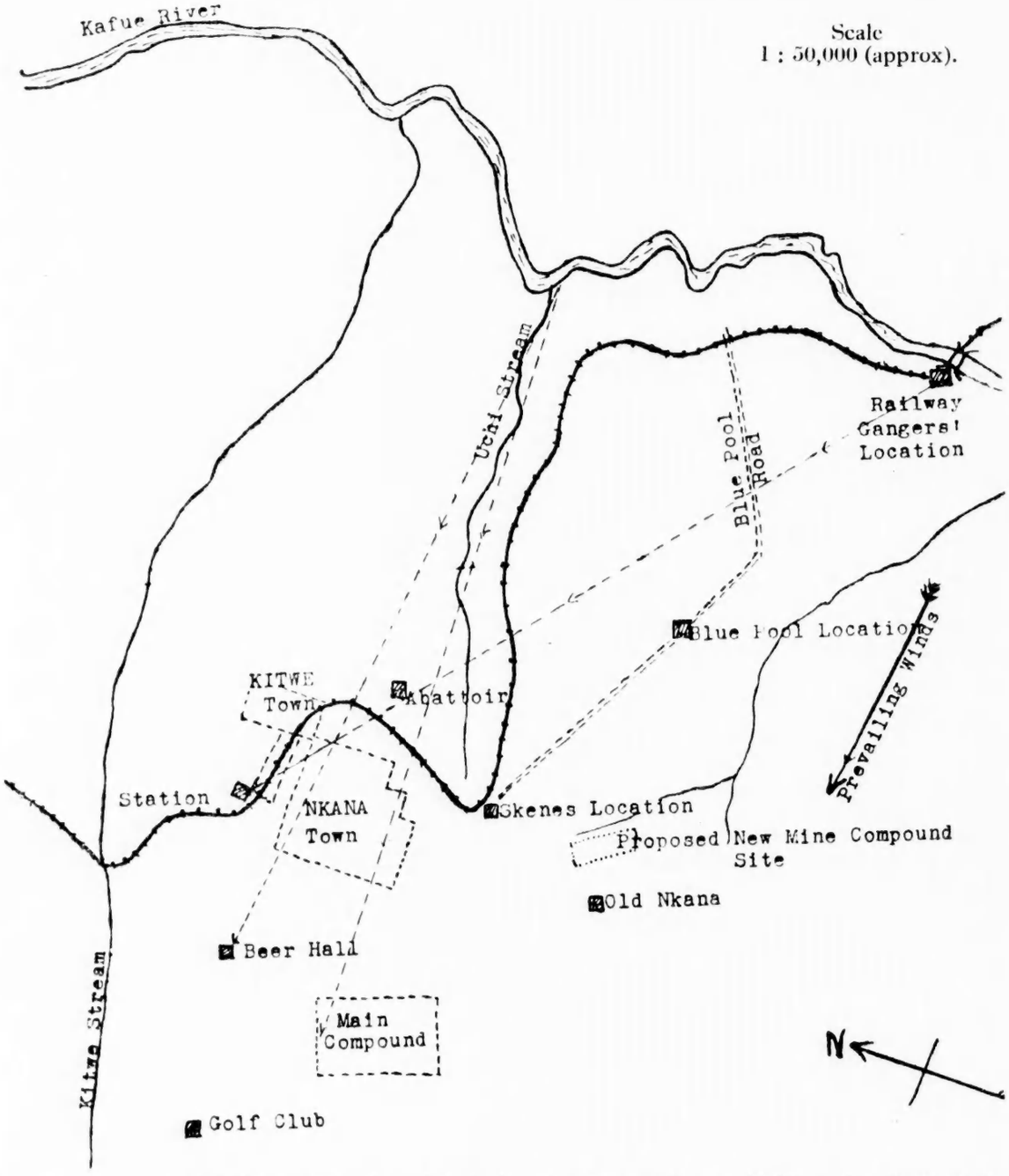

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3	160	150	94
		Approximate average	90

Here and elsewhere in this paper, therefore, after the figure representing the number of anophelines released, a second figure is given in parentheses to show the probable number of mosquitoes which were actually stained at the time of release.

Recaptured :

1 on following day at Kitwe ; distance 2·65 miles.

3 on second day at abattoir location ; distance 2·25 miles.

April 2nd, 1939 ; 5.30 p.m. Beaufort scale no. 3.

500 (450) *A. funestus* and *A. gambiae* released at the point where the Blue Pool Road crosses the Rhodesian Railway line ; stained with 2 per cent. aqueous solution of methylene blue.

Recaptured :

1 *A. gambiae* and 1 *A. funestus* on second day at Beer Hall ; 4·25 miles.

1 *A. funestus* on second day at abattoir location ; 2·25 miles.

1 *A. funestus* on second day at Blue Pool location ; 1·45 miles.

1 *A. gambiae* and 1 *A. funestus* on third day at N'Kana ; 2·45 miles.

2 *A. funestus* on third day at main compound ; 4·30 miles.

The following extract from a report, dated June 1st, 1939, to the Medical Officer of Health, N'Kana, will indicate the reason for further flight-tests on May 3rd :

' *Influence of Railway Gangers' Location, 4 miles S.E. and to Windward of N'Kana*

' This Location, erected temporarily to accommodate about 50 natives, plus women and children, employed ballasting the line to N'Kana, was put up in March, practically three months before it was discovered and controlled by insecticidal spray. In view of the possibility of infestation from this source, I immediately brought the Location under control by catching imagines and spraying the huts daily with an insecticide. This Location is situated about 50 yards to windward of Kafue River and between it and N'Kana.

' During the first fortnight when it was under control, 2,400 *Anopheles* were captured in 69 huts, averaging 200 per visit. Since control was introduced a remarkable decrease has been noted in the daily catches at the Abattoir Location (from 49·64 to 21·64) and at N'Kana (from 11·23 to 7·92).'

In passing, it should be noted that we were led to search for a location in this south-easterly direction by simple deduction, from the fact that during March, April and the beginning of May an increase in the incidence of malaria at N'Kana over the incidence anticipated pointed to a source of infection beyond our control-area. Obviously, since by adult control we were controlling all known localities within two miles of N'Kana, the increase during March, April and May might possibly be explained—in part, at least—by the presence of an uncontrolled location existing without our knowledge and providing a good focus of infection with (*a*) heavy infestation by *Anopheles* ; (*b*) high rate of carriers ; (*c*) high sporozoite-rate in the mosquito.

That this particular location was ideal in these three respects was borne out by the following facts :

(*a*) The *Anopheles* caught in 14 days numbered 2,400.

(*b*) The parasite-rate amongst 22 children examined was 73 per cent., and amongst 36 adults 65 per cent.

(*c*) The sporozoite-rate in *A. funestus* was 15 per cent.

The question which now arose, and which we set ourselves to answer, was whether this location could appreciably affect N'Kana, situated four miles away and across intervening bush 30 ft. high, even though it was to windward. (We have already noted how insecticidal spraying in the huts reduced the numbers of adults at the abattoir, $3\frac{3}{4}$ miles away, and at N'Kana, 4 miles away. But we were aware that this reduction might possibly be due to other influences, such as the tightening up of larval control at or about this time, or an alteration of the prevailing winds.) It seemed unlikely, but, in view of our previous results, possible ; so further flight-tests were carried out.

May 15th, 1939 ; 6.45 p.m., wind nil ; 7 p.m., wind nil ; 9 p.m., wind south-south-east, 8-12 miles per hour.

400 (360) *A. funestus* released 200 yards west (N'Kana-wards) of the gangers' location, 4 miles south-east of N'Kana.

Recaptured :

1, 18 hours later, at N'Kana station location ; 4·50 miles.

1, ,, ,, ,, at N'Kana town ; 4·00 miles.

Owing to an unfortunate mistake 58 stained mosquitoes were recaptured at the gangers' location on the day following release. It is probable that, had they been left undisturbed, they would have travelled farther afield and that more might possibly have been found in the vicinity of N'Kana.

Other flight-tests made during 1939 are recorded below.

May 3rd, to test flight against wind :

330 (297) *A. gambiae* and *A. funestus* released at a point 1 mile west of the golf club.

Recaptured :

1 *A. funestus* at north side of main compound ; 1·50 miles against wind.

1 *A. gambiae* at north side of Kitwe dambo ; 1·50 miles diagonally (30°) up-wind.

1 *A. funestus* in Kitwe town ; 3·25 miles against wind (probably carried by motor-car from the golf-club vicinity. De Meillon and Gear (1939) report serious infestation of anophelines by motor-car).

May 31st, 1939. Test to ascertain the possibility of infestation of N'Kana with anophelines from a proposed new compound site three-quarters of a mile south-east of N'Kana.

400 (360) *A. funestus* released at 5.50 p.m. ; wind at ground-level nil, at 300 ft., Beaufort scale no. 2, approximately 4-7 miles per hour.

Recaptured :

No.	Hours after Release	Locality	Distance	Direction to wind
1	18	N'Kana	1 mile	Diagonally across
1	18	Main compound north	$1\frac{1}{2}$,,	Down
4	42	N'Kana	$\frac{3}{4}$, 1, 1, $1\frac{1}{4}$,,	Diagonally across
1	68	Skenes	$\frac{1}{4}$,,	,, ,,
1	68	Kitwe	$1\frac{7}{8}$,,	,, ,,
2	90	N'Kana	$1\frac{1}{4}$, $1\frac{3}{4}$,,	,, ,,
1	240	Old N'Kana	$\frac{1}{4}$,,	Up-wind

The *funestus* taken after 10 days at Old N'Kana was the first one caught by us after a period longer than four days ; moreover, it was the only one in the test to fly apparently up-wind, though the distance covered was only a quarter of a mile. Seventeen observations from 0 to 90 hours after release, and at various times between 7 a.m. and 9.30 p.m., gave the following wind-directions : east, 4 times ; south-east, 5 times ; south-south-east, 8 times.

To complete the experiment, 400 (360) *A. funestus* were released on June 7th, 1939, at a point $1\frac{1}{2}$ miles south-west of N'Kana on a site which I considered more suitable for a new compound, i.e., it was not so potentially dangerous to European health. Time, 9.30 p.m. ; winds east to south-east, variable, Beaufort scale no. 3. *Recaptured* : nil.

One final observation : Boyd (1930) writes :

> ' Special circumstances appear to be necessary to render . . . long migrations possible, namely :
>
> ' (1) Heavy production, probably abnormally heavy.
>
> ' (2) A production area that permits this movement in a direction at right angles to the prevailing breeze.
>
> ' Some field workers have given much consideration to this question in deciding the radius in which malaria operations are to be conducted. . . . If the immigrants can find attractive places for oviposition within the area invaded, a considerable proportion will probably be tempted to remain, and consequently may later exert an appreciable effect on the local incidence of malaria. If such opportunities do not exist, they will be compelled to go far abroad as their ovaries mature, and will probably not return again.'

Boyd's first point, ' abnormally heavy production,' is undoubtedly furnished by the Kafue River near N'Kana ; but our observations tend to show that movement will be not only in a direction ' at right angles to the prevailing breeze,' but also down-wind.

As regards Boyd's remark that ' there is but slight or no evidence so far that such long-distance movement is a positive factor in malaria transmission if potential local breeding areas do not exist,' experience at N'Kana tends, I think, to show that long-distance immigration may be a positive factor whether potential breeding-places exist or not. The fact that imagines already infected near their source of production are travelling into a controlled area is sufficient to be a positive factor. Moreover, with the production-area abnormally heavy,

TABLE

Anopheline flight-tests at N'Kana, Northern Rhodesia

No. and species	Date and time	Wind	Where released	No. recaptured and where found	Distance covered, in miles	Hours after release	Position to wind when recaptured
1. 500 (450) *A. funestus* and *A. gambiae*	2.4.39; 5.30 p.m.	S.E. 8-12 m.p.h.	At junction of Blue Pool Road and railway	1 *funestus* and	4·25	48	Down
				1 *gambiae* at Beer Hall;	4·25	48	,,
				1 *funestus* at Blue Pool;	1·45	48	,,
				1 *funestus* at abattoir;	2·25	48	Diagonally across
				1 *funestus* and	2·45	72	Down
				1 *gambiae* at N'Kana;	2·45	72	,,
				2 *funestus* at main compound	4·30	72	,,
2. 500 (450) *A. funestus* and *A. gambiae*	2.4.39; 6 p.m.	S.E. 8-12 m.p.h.	At confluence of Uchi and Kafue River	1 *funestus* at Kitwe;	2·65	24	Down
				2 *gambiae* at abattoir;	2·25	48	,,
				1 *funestus* at abattoir	2·25	48	,,
3. 330 (297) *A. funestus* and *A. gambiae*	3.5.39; 6.30 p.m.	S.S.E. 4-7 m.p.h.	One mile W. of golf club	1 *funestus* at main compound;	1·50	18	Against
				1 *gambiae* at N. Kitwe dambo;	1·50	18	Right-angles
				1 *funestus* at Kitwe	3·25*	18	Against
4. 400 (360) *A. funestus*	15.5.39; 6.45 p.m.	S.S.E. 8-12 m.p.h.	Gangers' location, 4 miles S.E. of N'Kana	1 *funestus* at N'Kana;	4·50	18	Diagonally across: angle 45°
				1 *funestus* at N'Kana	4·00	18	Diagonally across: angle 30°
5. 400 (360) *A. funestus*	31.5.39; 5.50 p.m.	S.S.E. 4-7 m.p.h.	New compound site, S.E. of N'Kana	1 *funestus* at N'Kana;	1·00	18	Diagonally across
				1 *funestus* at main compound;	1·50	18	Down
				4 *funestus* at N'Kana;	0·75	42	Diagonally across
					1·00	42	,, ,,
					1·00	42	,, ,,
					1·25	42	,, ,,
				1 *funestus* at Skenes;	0·25	68	,, ,,
				2 *funestus* at N'Kana;	1·25	90	,, ,,
					1·75	90	,, ,,
				1 *funestus* at Kitwe;	1·80	68	,, ,,
				1 *funestus* at Old N'Kana	0·25	240	Against

**funestus* probably conveyed by motor car.

it seems hardly necessary for the imagines to return (to N'Kana, in this instance) after oviposition, to influence malaria, the influence being already felt *from their first visit.*

CONCLUSIONS

1. The half-mile radius, as a basis to fix the boundary for the area of anti-larval and anti-adult mosquito-control, in Northern Rhodesia is not valid where there is abnormal production, especially where the source of production is to windward or diagonally to windward.

2. *Anopheles funestus* have been demonstrated as travelling various distances up to 4·50 miles down-wind, 4·50 miles at an angle of 45° to wind, 1·50 miles at an angle of 30° up-wind, and 1·80 miles at right-angles to wind.

3. *Anopheles gambiae* have been shown to travel up to 4·25 miles down-wind, and 1·50 miles at right-angles to wind.

4. Adult mosquitoes travelling three or four miles into a larval-controlled area may, if already infected, affect the malaria incidence.

5. The method of combating such a menace as that outlined in paragraph 4 above appears to be (*a*) removal of the source of infection from proximity to production-area, or (*b*) adult control at the source of infection, and in larval controlled areas as a complementary measure.

It seems that, despite the ' satisfactory results ' of anti-adult control obtained by General Gorgas in Panama, Dr. Ross and Dr. De Meillon in South Africa, and a few other authorities elsewhere, this valuable aid to malaria-control has generally been lost sight of.*

6. Further research is needed on the subject of what may be termed ' immigrant malaria,' i.e., malaria conveyed by mosquitoes over a long distance.

ACKNOWLEDGEMENTS.—I should like to acknowledge my indebtedness to Dr. O. Hooper, F.R.C.S., Chief Medical Officer, N'Kana, for granting facilities for these tests. For encouraging the publication of this report, and for kindly correcting the manuscript, I am indebted to Dr. De Meillon, South African Institute for Medical Research, Johannesburg. I also gratefully acknowledge the assistance given me by Mr. K. Campbell and Mr. L. Bunner, Ph.L.T.A.C., of the European Hospital, N'Kana, the former for assistance with the flight-tests, the latter for examining the blood-slides.

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*After writing this paragraph, I received the November, 1939, issue of the *Transactions of the Royal Society of Tropical Medicine and Hygiene* (vol. 33, no. 3), in which I read with much pleasure that Colonel J. A. Manifold and Colonel S. P. James both advocate adult control (insecticidal spraying of huts, barracks, tents, etc.) as a valuable aid to anti-malaria measures in war-time (pp. 296-302).

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ATTEMPTS TO REDUCE ACQUIRED ATOXYL-RESISTANCE IN *TRYPANOSOMA GAMBIENSE*

BY

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(*Received for publication March 6th*, 1940)

Since Ehrlich's work in 1909, it has been known that trypanosomes susceptible to atoxyl can, by continued treatment with subcurative doses, be made so resistant to the drug that even the maximum tolerated dose has no appreciable effect on the trypanosomes in infected animals. As demonstrated by Yorke and Hawking (1932), passage through different species of laboratory animals does not affect this resistance, which persists even after cyclical development in Glossina (Yorke, Murgatroyd and Hawking, 1933).

Ehrlich (1909) believed that this acquired resistance is due to a reduced avidity of the receptor for the drug, as a result of which the drug does not unite with the protoplasm of the trypanosome. Yorke, Murgatroyd and Hawking (1931*b*) showed that, *in vitro*, tryparsamide does not enter tryparsamide-resistant trypanosomes—an observation in harmony with Ehrlich's theory. Without necessarily subscribing to Ehrlich's hypothesis, Yorke and his collaborators maintain that resistance is due to an impermeability of the trypanosome-membrane for the drug.

Drug-resistance, once acquired, appears to be a fixed and immutable character. Attempts to destroy it have all been unsuccessful, except those made by Citron (1931), who claimed to have abolished salvarsan-resistance in *Trypanosoma brucei* by injecting sodium thiosulphate intravenously into infected animals ; he states that after 17 passages in animals subjected to this treatment resistance was completely lost. Yorke, Murgatroyd and Hawking (1932*b*) could not confirm Citron's findings in *T. rhodesiense*.

It was at the suggestion of Dr. S. Adler that the present author attempted to reduce acquired resistance by damaging the trypanosome-membrane, since resistance is apparently due to a change in the properties of the membrane. And, although the results of the experiments described below are not consistent, it is perhaps desirable that they should be recorded, since they were carried on over the relatively long period of four years.

MATERIAL AND METHODS

The strain of *T. gambiense* used was obtained by Dr. Adler from the late Professor J. G. Thomson. At the time when experiments were begun the strain killed white mice 4–5 days after the appearance of trypanosomes in the blood. For mice the minimum curative dose of atoxyl, given in 1 per cent. solution, was 0·3 gm. per kilo. body weight, and from time to time the dose and its effects

were checked. In August, 1936, the trypanosomes changed spontaneously and the minimum curative dose was found to be 0·15 gm. per kilo. body weight ; in other words, the natural resistance had been reduced without any experimental interference.

The strain was made resistant by injecting subcurative doses of atoxyl into infected mice, followed by subpassage about 18 hours later. Successive passages were treated with gradually increasing doses of atoxyl, and after 4–7 months trypanosomes were not cleared from the blood by amounts of the drug approaching the minimum lethal dose (0·62 gm. per kilo. body weight). Moreover, Dr. R. Freund found that the strain had also become resistant to trypaflavine : while the minimum curative dose of trypaflavine was 0·02 gm. per kilo. body weight, mice infected with the atoxyl-resistant strain were not cured by the almost lethal dose of 0·03 gm. per kilo. body weight.

Several substrains originating from the parent stock were made resistant, and the resistance was found to be sustained during an observation-period of about four years.

PHYSICAL AGENTS

Mixtures of infected blood and saline kept for 3–24 hours at a temperature of –12° C. were found to be non-infective for mice.

Mixtures of infected blood and saline, after being kept for 2½ hours at 0° C., were inoculated into mice. After infection had been established, the trypanosomes were again kept at a temperature of 0° C. for 2½ hours. This process was repeated 10 times, but had no influence on atoxyl-resistance.

Mixtures of infected blood and saline were placed in a water-bath at a temperature of 50–52° C. : all trypanosomes were dead in 3–5 minutes.

After exposure for 1 hour at 49° C., trypanosomes were inactive but infective for mice. After repeating the process seven times atoxyl-resistance was still unaffected.

Mixtures of infected blood and saline were exposed in an open glass container to radiant heat from an electric radiator, the distance of the container from the source of heat being so adjusted that the temperature in the container was 49° C. After 50 minutes' exposure all the trypanosomes were dead. After 35 minutes' exposure mice were inoculated, and the process was repeated twice, but the atoxyl-resistance was not affected. After the third exposure, however, trypanosomes no longer appeared in the blood.

After exposure to a temperature of 49° C., followed by exposure to 0° C., trypanosomes were inactive but still infective. Repeating the process after two subpassages did not affect drug-resistance.

CHEMICAL AGENTS

In the following experiments the optimum concentration of various compounds was used, the optimum concentration being the concentration

necessary to inactivate the trypanosomes at 37° C. without destroying their infectivity for mice. As a result of subjecting trypanosomes to such treatment, the incubation-period for mice was prolonged in comparison with that of the untreated strain (up to 30 days).

Potassium hydroxide. Infected blood and an equal volume of 0·1 per cent. KOH in normal saline were incubated for 45 minutes. The trypanosomes became inactive. After incubation and subpassage five times, resistance was unaffected.

Potassium chloride. Five drops of infected blood plus 2 c.cm. of 1·5 per cent. KCl were incubated for 1–3 hours. The trypanosomes became inactive. Exposure and subpassage repeated five times did not affect drug-resistance.

Magnesium chloride. Five drops of infected blood plus 2 c.cm. of 1·5 per cent. $MgCl_2$ were incubated for 30 minutes. The trypanosomes became almost inactive. (After 40 minutes all the trypanosomes were dead.) Exposure and subpassage repeated five times did not affect resistance.

Tannic acid. Equal parts of infected blood and 0·2 per cent. tannic acid in normal saline were incubated at 37° C. for 3 minutes. The trypanosomes became inactive but were infective. Exposure and subpassage repeated seven times did not affect resistance.

Equal parts of infected blood and 0·3 per cent. tannic acid in 10 per cent. glucose were incubated for 60 minutes. The trypanosomes became inactive. Exposure and subpassage through mice repeated seven times did not affect resistance.

Sodium taurocholate. Equal parts of infected blood and 0·1 per cent. sodium taurocholate in saline were incubated for 60 minutes. The trypanosomes became almost inactive. Exposure and subpassage through mice repeated seven times did not affect resistance.

Urea. Equal parts of infected blood and 5 per cent. urea in saline were incubated for 65 minutes. The bodies of the trypanosomes became swollen; the flagella were active. Exposure and subpassage through mice repeated 11 times did not affect resistance. It is therefore obvious that damage to the body of the trypanosome, resulting in the swelling of the protoplasm, does not necessarily affect resistance.

Caffeine. Five drops of infected blood plus 10 drops of 1 per cent. caffeine puriss. in normal saline were incubated for 45–60 minutes. The bodies of the trypanosomes became swollen and inactive, but they remained infective. Exposure and subpassage repeated eight times did not affect resistance.

Sodium thiosulphate. Equal parts of infected blood and 10 per cent. sodium thiosulphate in saline were incubated for 30–45 minutes. The trypanosomes became inactive. Exposure and subpassage repeated seven times did not affect resistance.

Following the technique of Citron (1931), infected mice were inoculated intravenously with 1 c.cm. of a 2·5 per cent. solution of sodium thiosulphate, and the strain was subinoculated 16–24 hours later. Succeeding subpassages were treated in the same way, the degree of resistance being ascertained after each subpassage. Inoculation and subpassage repeated 20 times did not affect resistance.

Hypotonic solutions. (*a*) *Sodium chloride.* Five drops of infected blood and 1 c.cm. of 0·35 per cent. saline were incubated for 1 hour and inoculated into mice. Exposure and subpassage repeated five times did not affect resistance.

(*b*) *Glucose.* Equal parts of infected blood and 1 per cent. glucose in distilled water were incubated for 1 hour. Exposure and subpassage repeated five times did not affect resistance.

Hypertonic solutions. Solutions of potassium chloride, sodium chloride and calcium chloride were prepared isotonic with 10 per cent. glucose. One part of infected blood and two parts of hypertonic solution were incubated for 1 hour. KCl : the trypanosomes were inactive and non-infective. $CaCl_2$: the trypanosomes were destroyed and non-infective. NaCl : the trypanosomes were inactive ; only one subpassage was successful, and resistance was not influenced.

Glucose. The only method which appeared to reduce resistance was to incubate infected blood and 10 per cent. glucose in equal parts for various periods (series A : 50 minutes ; series B and D : 65 minutes ; series C : 110 minutes), to subpassage through mice, and to repeat the exposure to the solution. After exposure the trypanosomes were inactive but infective. The course of the experiments is shown in the table on page 49.

The time required to reduce resistance from a dose of 0·62 gm. (in the relatively few animals which survived this dose) to one of 0·3 gm. atoxyl per kilo. body weight was only $2\frac{1}{2}$ months, during which period the trypanosomes were subjected to hypertonic glucose up to 13 times. It is interesting to note the narrow margin during this period between the minimum curative dose and the minimum dose required to clear the blood temporarily of trypanosomes.

During the whole course of the experiment, control-experiments were carried out by incubating the parent strain with saline or 1 per cent. glucose ; neither process had any influence on resistance.

After the resistance of strain A had been reduced to normal, the strain was again made resistant to atoxyl in a dose of 0·62 gm. per kilo. body weight by the method used in the first experiment, i.e., by repeated exposure to subcurative doses. After resistance had been re-established, it was found impossible to reduce it experimentally by repeated exposures to 10 per cent., 15 per cent. and 20 per cent. glucose, chloralhydrate (1 : 1,000, 1 : 500), 20 per cent. sorbite, or 5 per cent. urea. The strain was maintained in guinea-pigs for three years, at the end of which period it was still resistant to a dose of 0·62 gm. per kilo. body weight in the few mice which survived this dose.

The positive results obtained in series A, B, C and D seemed to indicate the possibility of reducing artificially produced atoxyl-resistance in *T. gambiense*. The experiments were therefore repeated with the parent strain, but the results were varying and inconstant; the results obtained with strains A, B, C and D were not obtained again.

TABLE

	Series							
	A		B		C		D	
	Cured by	Not cured by	Cured by	Not cured by	Cured by	Not cured by	Cured by	Not cured by
In vitro treatment—								
1st	0·42	0·35	0·45	0·42	0·44	0·4	0·45	0·4
2nd	0·42	—	0·38	—	0·39	—	0·42	0·4
3rd	—	0·34	0·38	—	0·38	0·34	0·41	0·4
4th	0·4	0·38	—	0·37	0·38	—	0·38	—
5th	—	0·38	0·35	—	0·37	—	0·38	0·34
6th	0·35	—	0·34	0·3	0·35	0·33	0·35	—
7th	0·34	0·3	0·32	—	0·34	—	0·32	—
8th	0·32	—	0·3	—	0·3	—	0·3	—
9th	0·3	0·25	0·3	0·25	0·3	—	0·3	—
10th	0·3	—	0·3	—			0·3	—
11th	0·3	—	0·3	—			0·3	—
12th	0·3	—	0·25	—				
13th	0·3	—	0·25	—				

The figures in columns 2–9 represent gm. atoxyl per kilo. body weight.

In five series (F, G, H, I, K) treated with 10 per cent., 15 per cent., and 20 per cent. glucose for 180, 120 and 45 minutes respectively, the treatment being repeated after each subpassage for 10 more subpassages, no effect on resistance was observed.

In another series (E) resistance was reduced from a dose of 0·62 gm. to one of 0·43 gm. per kilo. body weight after 20 exposures to 10 per cent. glucose for 60 minutes with intervening subpassages. Further exposures to 10 per cent. glucose, 10 per cent. saccharose and 0·2 per cent. sodium taurocholate produced no further lowering of resistance.

In another series (R) resistance was reduced to a dose of 0·4 gm. per kilo. body weight after 20 treatments with 10 per cent. glucose for 60 minutes each. Beyond this point no further diminution of resistance was obtained.

The following experiments were carried out with strain R after resistance had diminished to 0·4 gm. per kilo. body weight.

Trypanosomes were incubated for 50 minutes in equal parts of 1 : 2,000 chloralhydrate and 10 per cent. glucose. After exposure and subpassage repeated 20 times resistance was unaffected.

Trypanosomes were incubated for 50 minutes in equal parts of 1 : 500 chloralhydrate and 20 per cent. glucose. After exposure and subpassage repeated 20 times resistance was unaffected.

After 17 exposures to 20 per cent. glucose for 20–40 minutes, mice were cured by 0·32 gm., and after 20 exposures by 0·3 gm., atoxyl per kilo. body weight. This diminution in resistance was only temporary, however, for after 10 further exposures the minimum curative dose was found to be 0·45 gm. atoxyl per kilo. body weight.

As was to be expected, staining, whether intra-vitam or by Giemsa, revealed no morphological differences between strains resistant and non-resistant *in vivo*.*

Chloralhydrate. Since partial or even complete removal of atoxyl-resistance had been achieved (though inconstantly) by exposing trypanosomes to a 10 per cent. solution of glucose, and since other solutions isotonic with 10 per cent. glucose had failed to influence resistance, it was thought that the aldehyde group in glucose might be responsible for the reduction of resistance.

In a long series of experiments a resistant strain was treated with chloralhydrate (1 : 2,000, 1 : 1,000, 1 : 500) alone or with equal parts of 10 per cent. or 20 per cent. glucose. After exposure and subpassage through mice repeated 50 times no influence on resistance was observed.

Alcohols. Exposures to 20 per cent. sorbite or glycerine in saline repeated 30 times with intervening subpassages had no influence on resistance.

ANTIMONY RESISTANCE

Strain A, after its resistance had been abolished and then re-established, as described above, was treated with gradually increasing doses of tartar emetic, until it became resistant to 0·045 gm. per kilo. body weight. Repeated exposures to 10 per cent. glucose, to chloralhydrate and to sorbite produced no effect whatever on the antimony- or the atoxyl-resistance of this strain.

SUMMARY

An attempt was made to reduce artificially produced atoxyl-resistance in *T. gambiense* by physical and chemical means.

In several series of experiments reduction was attained by repeatedly exposing trypanosomes *in vitro* to a 10 per cent. solution of glucose. Strains from which resistance was removed behaved like normal strains.

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*For vital staining the following method was found useful : 3 drops of a 5 per cent. solution of brilliant cresyl blue were added to 20 drops of an emulsion of trypanosomes, and the mixture was incubated for 5 minutes at 37° C. The trypanosomes, which at the end of this time were active, were then stained. Smears of the suspension were made after exposing a drop of the mixture in a formalin chamber.

We were unable to reduce resistance constantly : in some series of experiments a partial or temporary reduction of resistance was observed, but in most cases no effect was produced.

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THE COURSE OF *PLASMODIUM RELICTUM* INFECTION IN CANARIES AND THE TREATMENT OF BIRD AND MONKEY MALARIA WITH SYNTHETIC BASES

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The action of 1 : 11 undecane diamidine in monkey and bird malaria was reported on by Christophers and Fulton (1938) and the detailed course of *Plasmodium knowlesi* infection in rhesus monkeys was then fully dealt with. In the present communication the course of *P. relictum* infection in canaries has been similarly treated, as the information acquired over a period of years in regard to this infection is of some practical importance. This strain of bird-parasite was originally sent by Professor Raffaele in Rome to Sir Rickard Christophers in London in 1933 through the agency of infected mosquitoes, and has been maintained in canaries by means of blood inoculation and mosquito-infection since that date. It is somewhat more virulent than other strains of the same parasite in this country, and deaths among infected birds are not infrequent following blood inoculation, while in the case of mosquito-infected birds the death-rate is high. The virulent nature of the parasite has some possible advantages in assessing the value of anti-malarial drugs. The incubation-period is short and is followed by an acute phase with high parasite level. A long chronic stage results in which the blood is still infective, but parasites are not visible on microscopic examination and relapses are not common. Outside the body of the host it appears to be a delicate parasite and does not survive well *in vitro*.

The present investigations with amidine compounds were made on bird and monkey malaria at Professor Yorke's request, as he had had some successes

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with amidines against other organisms (Lourie and Yorke, 1939). All the tests of anti-malarial action were made with blood-inoculated animals, and admittedly may differ from infections naturally acquired.

These new compounds, in which the amidine groups are attached to benzene rings joined by inert carbon or carbon-oxygen chains, differ from other anti-malarials hitherto employed in therapeutics. The action of some simple bases against bird malaria is recorded at the same time.

THE NORMAL COURSE OF *P. RELICTUM* INFECTION

Day of First Appearance of Parasites

Transmission was carried out by injection of 0·1 c.cm. of citrated blood into the pectoral muscles of a canary from a stock bird with parasites in the peripheral circulation. The time of appearance of parasites varied from 2 to 10 days (average 5·6 days) and was influenced by the numbers of parasites injected. The incubation-periods for a number of birds, where the first day of appearance of parasites was accurately noted, are given in Table I.

Table I

Showing incubation-periods in blood-infected canaries

Day of first appearance of parasites	No. of birds	Mean period, in days
2	1	
3	6	
4	36	
5	60	5·6
6	36	
7	26	
8	15	
9	3	
10	4	
Total	187	

The resulting infection varies in intensity, as indicated by some typical counts given in Table II, in which the number of parasites per 100 microscopic fields is recorded. Approximately 400 red cells were present in each field at the start of the infection, but the number decreased progressively as anaemia became more marked. Parasites when first seen number 1 per 100 or more fields, and they can be seen in the peripheral blood for 2–3 weeks. The proportion of deaths in 100 birds was from 10 to 15 per cent., presumably due to the

malaria, as the anaemia becomes very pronounced. Bird 1168, for example, on the 20th day had 3,500 parasitized cells and 4,200 non-parasitized cells per 100 fields ; it died two days later.

TABLE II

Showing the course of infection following blood inoculation : number of parasites per 100 microscopic fields

Bird no.	Days following inoculation								
	4	5	6	7	8	9	10	11	12
1111	0	1	12	300	1,500	—	—	100	—
1125	—	—	—	—	32	400	1,300	1,900	D
1127	0	—	—	300	1,200	2,300	700	200	—
1180	3	28	—	—	4,900	6,100	6,200	—	5,700
1240	—	8	88	1,200	900	2,600	2,000	D	
1274	—	40	300	1,100	1,300	—	1,900	—	6,000 D
1085	0·5	—	32	—	2,000	—	—	—	8
1102	—	1	—	24	—	400	—	—	1
1148	0·5	—	—	32	—	600	—	—	1
1289	—	0	9	72	112	—	—	—	0·5
1357	0	0·5	1	—	52	D			
1440	0	0·5	8	0	4	8	0	0	0
1168	—	—	700	—	4,000	—	5,500	—	—

D = died.

The above counts give only a very approximate idea of the rate of parasite-increase, which is of a stepwise character, with a gradual fading-out if the bird survives. The first six cases are representative of more severe infections, while the six which follow are milder in character.

Multiple infections, up to three or four per red cell, are common, and the red cell nucleus is often displaced. Relapses are rare, but bird 1105 died with 10,000 parasites per 100 fields following such a relapse, after parasites had been present in the peripheral blood for 16 days. Bird 1209 had 48 parasites per 100 fields on the 22nd day.

In the case of mosquito-infected birds, the number dealt with is much smaller (see Table III). As is shown there, the inoculation-period of birds infected by this means was slightly longer than in those infected by blood inoculation. The number of mosquitoes biting did not appreciably influence the length of this period (see Table IV).

TABLE III

Showing length of incubation-period following mosquito-infection

Day of first appearance of parasites	No. of birds	Mean period, in days
5	1	
6	7	
7	7	7·0
8	4	
9	1	
10	1	
Total	21	

The character of the mosquito-infection is similar to that of blood-infected birds, but the mortality amongst the birds was much greater, and a high percentage of deaths resulted when the infection was allowed to run unchecked, as shown in Table IV.

TABLE IV

Showing the course of mosquito-infection in canaries : number of parasites per 100 microscopic fields

Bird no.	No. of mosquitoes biting	Days following inoculation								
		6	7	8	9	10	11	12	13	14
1064	1	0	+	56	D					
1065	1	0	+	40	—	1,000	—	—	100	D
1074	14	2	—	132	—	100	—	—	800	1,400 D
1075	9	2	—	100	—	100	D			
1082	39	0·5	4	60	Killed					
1083	15	1	24	—	2,400	D				
1172	3	20	—	—	—	—	—	2,200	D	
1445	20	0	0·5	—	—	—	300	—	—	120

D = died.

GAMETOCYTES

Gametocytes were generally noted a day or two after the asexual forms, and relatively early in the infection. The maximum number found in each field was two or three at the height of infection, although in one case six were seen. The regular increase in numbers which occurs in the asexual forms was not apparent. In some infections of small intensity gametocytes were consistently absent. The data regarding the appearance of gametocytes is given in Table V for blood-inoculated birds.

TABLE V

Showing day of appearance of gametocytes after blood inoculation

Day of first appearance of gametocytes	No. of birds	Mean period, in days
4	3	
5	4	
6	21	
7	24	
8	37	7·9
9	16	
10	11	
11	7	
12	3	
Absent	16*	
Total	142	

* This number has not been considered in calculating the mean.

The female is round, with dark-blue cytoplasm and a central dot of chromatin. The pigment is coarse and distributed. The red cell nucleus may be absent or displaced, and free forms outside the red cell are commonly seen in stained preparations. The male is round, with brick-red cytoplasm if well stained. The pigment is distributed, and not easily observed in a well-stained specimen. Chromatin is present throughout the cell and cytoplasm is scanty in amount. The forms are very often seen free in a stained preparation.

PERIODICITY

The duration of the schizogony-cycle is approximately 24 hours, and at the same hour each day the same stage is seen. Thus, at 10 a.m. half- to three-quarters-grown forms are present and, if faintly stained, have a small amount of pigment visible, generally at the edge of the parasite and not distributed; in half-grown forms it is rare. At 2 p.m. presegmenting forms with three or more dots of chromatin are present; and at 5 p.m. are seen segmenting forms with up to 20 or more dots of chromatin, which are very difficult to count, and a dot of clumped pigment, generally central. From 8 to 10 p.m. ring-forms with a single dot of chromatin make their appearance. There is thus regularity in the time of appearance of the different stages. Certain arbitrary stages of development were selected, into which the parasites could reasonably be grouped. Differential counts were then made from stained preparations at fixed times over a number of days, to determine the duration of the schizogony-cycle more accurately, and 100 parasites were counted at each time selected. The actual figures for the counts are given in Table VI, and the percentages of each

TABLE VI

Showing the percentage of parasites at different stages of development observed at definite times over a number of days, together with the number of non-infected red cells and gametocytes present with 100 asexual parasites

BIRD No. 1425

Date	Time	Segmenting and presegmenting forms	Half- to full-grown forms	Rings and quarter-grown forms	Gametocytes		Non-infected red blood cells
					M.	F.	
12.1.39	2 p.m.	77	23	0	1	3	1,375
	3 p.m.	86	13	1	2	2	1,056
	4 p.m.	86	14	0	7	5	1,098
	10 p.m.	2	0	98	1	3	243
	11 p.m.	0	0	100	1	7	406
13.1.39	10 a.m.	5	81	14	7	5	1,054
	11 a.m.	13	74	13	6	14	1,080
	12 noon	19	75	4	7	9	1,093
	2 p.m.	54	44	2	4	10	929
	3 p.m.	79	21	0	7	6	1,332
	4 p.m.	90	10	0	7	12	1,186
	10 p.m.	3	0	97	2	7	324
	11 p.m.	0	0	100	2	4	273
14.1.39	10 a.m.	3	89	8	2	4	450
	11 a.m.	26	73	1	3	10	789
	12 noon	32	68	0	5	7	1,014
	2 p.m.	52	38	0	3	7	909
	3 p.m.	68	32	0	7	2	814
	4 p.m.	88	12	0	3	6	796
	10 p.m.	11	0	89	5	10	432
			BIRD No. 1426				
16.1.39	5 p.m.	71	29	0	1	4	19,058
	10 p.m.	1	0	99	1	4	1,715
17.1.39	10 a.m.	3	97	0	1	2	3,680
	5 p.m.	86	14	0	15	16	5,520
	10 p.m.	2	0	98	5	4	949
18.1.39	10 a.m.	0	100	0	4	5	1,834
	5 p.m.	61	39	0	2	15	2,644
	10 p.m.	4	0	96	9	14	905
19.1.39	10 a.m.	0	79	21	3	4	1,218
	5 p.m.	77	23	0	6	10	1,855
	10 p.m.	24	1	75	6	25	1,500
20.1.39	10 a.m.	0	100	0	4	6	1,115
	5 p.m.	81	19	0	2	4	2,286
	10 p.m.	3	2	95	4	6	603
21.1.39	10 a.m.	1	94	5	1	1	607
	10 p.m.	14	1	85	4	6	480
22.1.39	10 a.m.	0	77	23	0	2	627
	5 p.m.	62	38	0	2	6	883
	10 p.m.	22	2	76	5	16	533

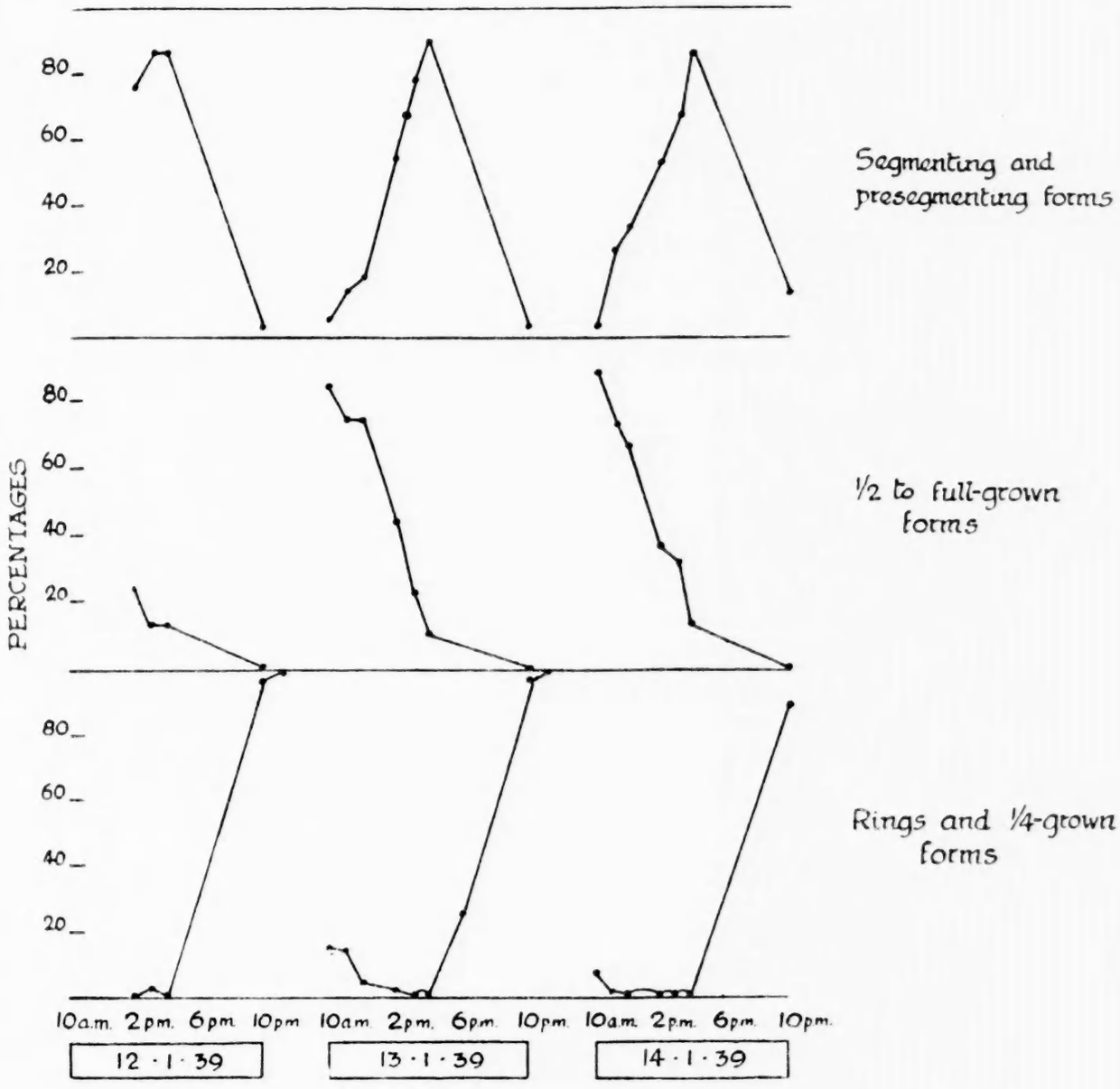

CHART showing the different stages of development of *P. relictum* plotted in percentages against the times at which observations were made.

stage of development are plotted against the time of observation in the chart. Actually, the counts as made were much more elaborate than recorded, but they are given as in Table VI for the sake of simplicity. From the results obtained it would appear that the schizogony-cycle approximates very closely to 24 hours. The infections as noted from the table are of increasing severity, but it is difficult to relate the factor of increase with the number of dots of chromatin seen in the segmenting forms, and presumably with merozoite formation, from these films of the peripheral blood.

AMIDINES AND OTHER BASES

Effect against Bird Malaria

Canaries of known weight were infected by blood inoculation into the pectoral muscle, and the drug, made up in distilled water and calculated on the basis of a 20 gm. bird, was given intraperitoneally or by mouth 4 hours later. Dosage was repeated on five successive days. Toxicity was determined beforehand by a similar number of injections of the drug in question. Relevant details are recorded in the tables relating to the therapeutic action of the different drugs.

In the case of 4 : 4′-diamidino stilbene, the toxic intraperitoneal dose when repeated was found to be 0·3 mgm. or rather less, and birds were unaffected by four or five times this dose by mouth. 4 : 4′-diamidino-1 : 5-diphenoxy pentane was less toxic, and repeated doses of 0·4 mgm. intraperitoneally were well tolerated. The largest dose of this drug administered by mouth was 1 mgm.

2-amino-5-diethylamino pentane, $CH_3CH(NH_2)(CH_2)_3N(C_2H_5)_2$, an aliphatic base, was prepared because of its relation to plasmoquine, in which one of the hydrogen atoms of the amino group is replaced by 6-methoxy quinoline. Repeated 10 mgm. doses intraperitoneally, and double this dose by mouth, were well tolerated by canaries.

Tetraamyldiaminodecane, kindly supplied by Dr. F. L. Pyman, F.R.S., was tolerated in repeated doses of 1·25 mgm. intraperitoneally and of 5 mgm. by mouth.

In the case of quinine, 5 mgm. is well tolerated by mouth, while 10 mgm. gives rise to incoordination.

The therapeutic results obtained with the different drugs in *P. relictum* infections of canaries are shown in Table VII.

TABLE VII

Showing the effect of various drugs on *P. relictum* infection in canaries

4 : 4′-DIAMIDINO STILBENE

Bird no.	Dose in mgm. per 20 gm.	Route	Day of first appearance of parasites
1428	Control		6
1436	,,		4
1437	,,		8
1430	6 × 0·6	Mouth	5
1432	,,	,,	7
1433	6 × 0·2	Intraperitoneal	8
1434	,,	,,	7
1439	Control		5
1440	,,		5
1443	6 × 0·9	Mouth	6
1444	,,	,,	5
1486	Control		Died negative 5th day
1487	,,		6
1483	5 × 0·2	Intraperitoneal	Died negative 5th day
1484	,,	,,	5
1485	6 × 0·1	,,	6
1488	,,	,,	8

4 : 4′-DIAMIDINO-1 : 5-DIPHENOXY PENTANE

1456	Control		6
1457	,,		5
1458	6 × 0·4	Intraperitoneal	Died negative 12th day
1459	,,	,,	,, ,, 16th ,,
1460	6 × 0·5	,,	,, ,, 8th ,,
1461	,,	,,	,, ,, 8th ,,
1462	6 × 1·0	Mouth	,, ,, 18th ,,
1463	,,	,,	11
1464	Control		5
1465	,,		5
1466	6 × 0·3	Intraperitoneal	Died negative 20th day
1467	,,	,,	Killed negative 15th day
1468	6 × 0·2	,,	21
1469	,,	,,	Still negative 29th day
1486	Control		Died negative 5th day
1487	,,		6
1489	6 × 0·1	Intraperitoneal	4
1490	,,	,,	6
1493	Control		5
1498	,,		7
1496	3 × 0·1	Intraperitoneal	9
1497	,,	,,	5

TABLE VII (*Continued*)

2-AMINO-5-DIETHYLAMINO PENTANE

Bird no.	Dose in mgm. per 20 gm.	Route	Day of first appearance of parasites
1366	Control		4
1367	,,		5
1368	,,		5
1369	,,		5
1358	6 × 10	Intraperitoneal	8
1359	,,	,,	8
1360	,,	,,	4
1361	,,	,,	10
1362	6 × 20	Mouth	10
1363	,,	,,	16
1364	,,	,,	Remained negative
1365	,,	,,	9
	TETRAAMYLDIAMINODECANE		
1446	Control		5
1447	,,		8
1448	4 × 1	Intraperitoneal	6
1449	,,	,,	8
1450	4 × 2	Mouth	5
1451	,,	,,	6
	QUININE HYDROCHLORIDE		
919	5 × 5	Mouth	13
920	,,	,,	12
921	,,	,,	14
922	,,	,,	13
923	,,	,,	14
924	Control		7

From the results recorded in the above table it will be seen that 4 : 4′-diamidino stilbene is unable to delay the appearance of parasites in the peripheral blood in doses near the maximum. On the other hand, 4 : 4′-diamidino-1 : 5-diphenoxy pentane is effective in well-tolerated doses, but the therapeutic index is small. It is of interest that 2-amino-5-diethylamino pentane should possess anti-malarial properties, in view of its relationship to plasmoquine; its therapeutic index is, however, small, and large doses are necessary for treatment. Tetraamyldiaminodecane is of no interest as an anti-malarial. The experiment with quinine is given for purposes of comparison. The latter base has definite anti-malarial properties in *P. relictum* infections.

Effect against Monkey Malaria

P. knowlesi infections in rhesus monkeys follow a regular course, with death occurring in untreated animals about the fifth day after the first appearance of parasites in the peripheral blood. This type of infection is most useful in therapeutic studies. After the primary attack other attacks follow, which, although milder, may also prove fatal. The saving of life in the primary attacks is one criterion for the efficacy of a drug. After treatment of an attack an aparasitic interval may follow, and the length of this interval following the primary attack also serves as a guide to the value of a drug. In the present series of experiments, the total period of infection has not been considered, as animals were generally killed for some other purposes.

A completely effective drug would not only cause the disappearance of the parasites from the blood, but would render such blood non-infective on inoculation into susceptible animals, prevent relapses on splenectomy, and render the animal again susceptible to reinoculation. In the case of monkey no. 164, which received two doses of 4 : 4′-diamidino stilbene, parasites disappeared and failed to return when the animal was splenectomized a few weeks later. This result was quite exceptional in our experience of a large number of cases treated with quinine or atebrin.

The two amidine derivatives already mentioned were used in the tests. It was found that both were equally well tolerated by monkeys. Ten mgm. per kilo. given intravenously on three successive days gave rise to yawning and sickness, and caused the animals to lie down. Recovery was, however, rapid in each case. Five mgm. per kilo. by the same route was tolerated without any symptoms. It was regarded as a safe dose.

The intravenous route was employed therapeutically and the drugs were generally administered early in the attack. They clearly have an effect on *P. knowlesi* parasites, and all the animals survived. The parasites appeared to be directly affected, for cytoplasm stained poorly and the parasites were ragged and vacuolated ; those affected, however, disappeared but slowly from the blood. The animals did not appear to be ill during the infection. The oxygen-combining power of the blood was not affected by a concentration of drug much higher than that employed therapeutically. The measurements were carried out in Barcroft manometers as previously described (Christophers and Fulton, 1938). The inhibition of oxygen uptake—a measure of *in vitro* activity of the drug—is also recorded for each drug, along with the inhibition values previously obtained for quinine under the same conditions.

One prophylactic experiment was carried out with each drug. Four consecutive daily doses of 2·5 mgm. per kilo. were injected into each animal, two being intravenous and two intramuscular, and the animal was then inoculated with infected blood on the day of the last injection. The drug failed to prevent infection, but the incubation-period was somewhat longer than normal.

From the data given below it is seen that both drugs have a definite action against *P. knowlesi* in monkeys if given intravenously, and all animals were saved.

TABLE VIII

Showing the results of treatment of *P. knowlesi* infection in monkeys with (1) 4 : 4′-diamidino stilbene and (2) 4 : 4′-diamidino-1 : 5-diphenoxy pentane

(1) 4 : 4′-DIAMIDINO STILBENE

Monkey no.	Weight in kilo.	Day after appearance of parasites	Dose, in mgm. per kilo.	Parasites per 100 fields	Result
162	3·6	3	5	200	Aparasitic interval of 2 months, except for one positive examination
		4		1,500	
		5	5	2,300	
		6		48	
		7		0	
164	3·0	1	5	10	Remained negative even on splenectomy
		3	5	400	
		4		3	
		5		9	
		6		0	
166	3·2	2	5	60	Short aparasitic interval. Low-grade infection for 4 months followed
		4	5	3,000	
		5		24	
		7		0	
		22	5	2,800	
		23		12	
159	4·5	24	5	10	Previously treated with 3 doses of atebrin : poor result. Mild infection after splenectomy
		25		34	
		26		0	
(2) 4 : 4′-DIAMIDINO-1 : 5-DIPHENOXY PENTANE					
163	3·0	2	5	12	Low-grade infection for 4 months followed
		4	5	400	
		5		48	
		6		0	
		13	5	6,000	
		16		0	
165	3·0	3	5	10	Low-grade infection for 3 months. Second dose of drug given ; remained negative 2 months
		4		0	
		11		2	
		99	5	2,000	
		101		0	
168	4·5	2	5	200	Low-grade infection followed for 2 months
		3		300	
		4	5	700	
		8		4	
		11	5	2,700	
		14		1	
		18	5	40	
		21		0	
172	3·7	2	5	32	Failed to cure infection. Atebrin given
		3	intra-	300	
		4	muscularly	4,700	

Following incubation of fresh blood with these drugs at a molecular concentration of 0·002 for 4 hours at 37° C., no change could be detected in the absorption-bands by means of a comparison spectroscope.

The oxygen-combining capacity of blood incubated for 2 hours under the same conditions was found to be unaltered when measurements were made in Barcroft manometers.

The following results were obtained on measuring the inhibition of oxygen uptake by these drugs on a 1 : 10 suspension of parasites. The corresponding values obtained previously for quinine are included for comparison. Both amidines are powerful inhibitors *in vitro*.

TABLE IX

Showing the inhibition of oxygen uptake in *P. knowlesi* suspensions in presence of various drugs

Molecular concentration of drug	4 : 4′-diamidino stilbene	4 : 4′-diamidino-1 : 5-diphenoxy pentane	Quinine
0·0000001	4·4	0	—
0·000001	23·2	6·0	—
0·00001	54·4	38·6	3·3
0·0001	62·5	68·6	16·3
0·001	75·4	78·7	46·3

DISCUSSION

From the data given it is obvious that the aromatic amidines tested (4 : 4′-diamidino stilbene and 4 : 4′-diamidino-1 : 5-diphenoxy pentane) have definite anti-malarial properties in *P. knowlesi* infections. They were able to save life in this otherwise fatal infection, although they act somewhat more slowly than atebrin. In the case of the stilbene derivative, the aparasitic interval following the primary attack was of approximately the same duration as with atebrin; the phenoxy-pentane derivative was less effective. The therapeutic index of each is small. Relapses occurred in all animals except no. 164, which appeared to have been sterilized. Such a result has never been previously obtained by us. The total period of infection could not be compared with that for atebrin, as the animals were killed for other purposes. The number of animals treated was relatively small.

In the case of bird malaria, the stilbene derivative was inactive. The phenoxy-pentane derivative delayed the appearance of parasites for a considerable time when given in doses which were well tolerated. The same is true of the aliphatic base 2-amino-5-diethylamino pentane, which corresponds to the side-chain in plasmoquine.

SUMMARY

The course of *P. relictum* infections in canaries is described from observations made on several hundred birds.

The incubation-period, course and intensity of the infection, mortality, periodicity and time of appearance of gametocytes are recorded for blood-inoculated and, in some cases, for mosquito-infected birds.

The action of various bases against bird and monkey malaria is also given. It has been shown that the new aromatic amidines have definite anti-malarial properties in *P. knowlesi* infections of monkeys. 4 : 4′-diamidino-1 : 5-diphenoxy pentane is also active against bird malaria.

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STUDIES IN CHEMOTHERAPY*

XXIV.—CHANGES IN THE BLOOD PRODUCED BY ADMINISTRATION OF 4 : 4′-DIAMIDINO STILBENE

BY

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(*Received for publication April 17th*, 1940)

In a previous communication in this series dealing with trypanocidal amidine compounds (Devine, 1938), observations were recorded on the toxic effects of graded doses of undecane diamidine on the liver and kidneys of the rabbit and of man, as determined by analysis of the blood and urine. The main purpose of that study was to establish with some certainty the highest dose of the drug which could be administered to patients with complete safety. More recently, Lourie and Yorke (1939) have shown that some of the aromatic amidines also possess pronounced trypanocidal activity *in vitro* and exhibit striking activity against trypanosomal and certain other protozoal infections. The present note records an examination of the toxic effects of one of the most promising of these amidines, viz., 4 : 4′-diamidino stilbene. Quantitative analyses were confined in this case, however, to the blood (using the same methods as before), since previous experience showed that the anorexia and nausea following administration of this type of compound make interpretation of the urinary nitrogen balance very difficult.

According to the limited data supplied at the time by Dr. E. M. Lourie on the toxicity of this compound, intravenous injection of 30 mgm. per kilo. into each of three rabbits caused death within 1–2 minutes, whereas doses of 20 mgm. per kilo. were not generally lethal. In Tables I–III are recorded the variations in blood sugar and blood urea of normal fasting rabbits after graded doses of diamidino stilbene given by intravenous injection. It is to be noted that administration of this compound to rabbits in high doses generally made bleeding from the ear difficult and slow, even with stimulation, and that this probably accounts for otherwise insignificant variations in the blood sugar level.

* This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

Experiments I and II. Injection of 15 mgm. per kilo.

Experiment I was conducted for a period of over three days, with a short feeding-period on the routine diet at one point, but the animals ate practically nothing and were again fasted before the next observation was made. The blood sugar was not significantly affected, but the blood urea rose by some 50–100 per cent. within a few hours, and then fell to normal within less than three days.

Table I

Blood analyses after injection of 15 mgm. per kilo. of diamidino stilbene.

Rabbits. P, ♀, 1·9 kilo. Collapsed on injection, but soon recovered.
B, ♀, 2·4 kilo. Injection tolerated well.

Time after injection	Sugar, mgm. per cent.		Urea, mgm. per cent.	
	P	B	P	B
Control	108	114	40	55
2½ hours... ...	88	123	57	66
5 „	93	101	65	70
8 „	97	121	77	77
10 „	113	113	74	75
29 „	105	124	64	70
33 „	—	—	66	69
53 „	123	112	43	48
77 „	—	—	33	33

In a repetition of this experiment conducted without a break for a period of 54 hours with a fresh pair of rabbits, both animals tolerated the dose well and showed roughly the same metabolic effects as before. Somewhat greater increases in blood urea were observed (from 35 to over 100 mgm. per cent., and from 36 to 68 mgm. per cent. respectively), but the blood sugar level remained constant.

Experiment III. Injection of 25 mgm. per kilo.

Each of two rabbits given 25 mgm. per kilo. of diamidino stilbene intravenously collapsed almost immediately, but appeared to recover within less than an hour. One survived over an observation-period of several weeks, but the other, showing more severe metabolic disturbances, died 10 days after the injection.

TABLE II

Blood analyses after injection of 25 mgm. per kilo. of diamidino stilbene.

Rabbits. W, ♂, 1·6 kilo. Collapsed on injection, but completely recovered.
R, ♀, 2·3 kilo. Collapsed on injection, appeared to recover, but died 10 days after injection.

Time after injection	Sugar, mgm. per cent.		Urea, mgm. per cent.		Amino-N, mgm. per cent.	
	W	R	W	R	W	R
Control...	123	111	36	51	9·3	6·3
2 hours	213	200	56	79	—	—
4½ „	125	167	74	112	8·2	6·7
7½ „	104	105	87	132	7·8	6·7
11 „	105	114	100	143	8·4	—
25 „	122	123	108	176	8·1	6·1
2nd day after injection			97	154		
3rd „ „ „			78	145		
5th „ „ „			52	>100		
8th „ „ „			47	>100		
10th „ „ „			Survived	Dead		

In contrast to the effects obtained with smaller doses, both animals showed a marked and rapid rise of almost 100 per cent. in the blood sugar within two hours after injection, with an almost equally rapid fall to normal (Table II). After this hyperglycaemic phase there was no further variation or suggestion of a hypoglycaemia over a further period of 20 hours. There was no significant change in the blood amino-N level, measurements of which were made simultaneously as a further criterion of possible liver damage. This is generally the case when the effect on the liver is insufficient to cause either a *prolonged* hyperglycaemia or, more especially, hypoglycaemia. This transient stimulation of liver glycogenolysis was accompanied by a profound and much more seriously prolonged interference in kidney function. In each case the blood urea rose rapidly to a maximum within a little more than one day; but, whereas the less seriously affected animal showed a gradual recovery to almost normal figures within eight days (and survived the toxic effects successfully), the other still showed a 'plateau' effect with blood urea considerably above 100 mgm. per cent., and died two days later (10 days after the injection).

EXPERIMENT IV. INJECTION OF 27·5 MGM. PER KILO.

In the guanidines and the related amidine compounds, hypoglycaemia is in general attained only by lethal doses, when these are such as to permit of a survival period of at least several hours for observation. The 'classification'

of such compounds as being hyperglycaemic or hypoglycaemic may depend to a very great extent on the careful grading of the dosage and on other variable factors, such as the liver-glycogen content. This was made evident in the previous study on undecane diamidine (Devine, 1938). In an effort to obtain a state of hypoglycaemia with diamidino stilbene, a still larger dose of 27·5 mgm. per kilo. was injected into two fresh rabbits. One animal collapsed and died within five minutes, and the other (rabbit A), observations on which are recorded in Table III, collapsed immediately, but made a permanent recovery.

TABLE III

Blood analyses after injection of 27·5 mgm. per kilo. of diamidino stilbene.

Rabbits. One animal collapsed and died within 5 minutes.
A, ♂, 1·3 kilo. Collapsed, but made a permanent recovery.

Time after injection	Sugar, mgm. per cent.	Urea, mgm. per cent.	Amino-N, mgm. per cent.
Control	117	32	7·5
3 hours	113	59	8·0
4 ,, ...	104	66	8·0
8 ,, ...	122	73	7·2

This animal proved highly resistant to the toxic effects which might be expected from such a high dose ; there was no evidence of hepatic injury and the rise of blood urea was not much greater than for rabbit P (experiment I), which had collapsed (with ultimate recovery) after a much smaller dose. As four experiments involving eight injections of single high doses had failed to bring about a hypoglycaemic phase, no further tests were made along these lines.

EXPERIMENT V. INJECTION OF REPEATED SMALL DOSES

In order to test the possibility that repeated small doses, such as would be given clinically, might lead to cumulative injury, each of two rabbits was injected with 5 mgm. per kilo. of diamidino stilbene on six successive days. Analyses were made 5 hours after injection on the first, fourth and sixth days. No significant changes in blood sugar or urea were observed.

Substantially, therefore, the results of administration of diamidino stilbene to rabbits resemble those due to undecane diamidine, damage to the kidneys being prior to, and independent of, hepatic injury. Successive small doses are tolerated well, without any apparent effect. Nitrogen retention is effected by a dose of 15 mgm. per kilo. (and probably by less), which is tolerated fairly well without alteration in the blood sugar. Higher doses, which are tolerated with

difficulty or which eventually prove lethal, cause an increased and prolonged nitrogen retention, accompanied by hyperglycaemia of relatively short duration. No definite conclusion can be drawn as to whether the inability to induce hypoglycaemia in a limited number of experiments indicates any significantly different response in the animal, as compared with that towards undecane diamidine.

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THE TREATMENT OF EARLY CASES OF NIGERIAN TRYPANOSOMIASIS WITH 4 : 4′-DIAMIDINO STILBENE

BY

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(*Received for publication May 29th*, 1940)

In June, 1939, Dr. R. D. Harding, who had been using 4 : 4′-diamidino stilbene at Gadau in Northern Nigeria, communicated with me at the request of the Deputy Director of the Sleeping Sickness Service, Nigeria. I was asked to treat, with the limited supply of the drug then available, cases of trypanosomiasis showing slight or no involvement of the central nervous system.

A sleeping sickness team was then about to begin a mass survey and treatment campaign in the Dass and Toro Districts of the Bauchi Province of Northern Nigeria. In these areas tsetse is not abundant : *Glossina morsitans* has not been identified, *G. palpalis* is most common in the west (Toro District), and *G. tachinoides* in the east (Dass District) where the streams are less densely shaded.

In neither Dass nor Toro had a mass survey previously been made, and few cases of sleeping sickness from these districts had ever attended for treatment at the central hospitals in Bauchi and Jos. A high incidence of the disease was, therefore, not expected. Latterly a few cases, cast out by relatives, had been cared for by the staff of the Sudan Interior Mission, and of these some had been sent to Bauchi for treatment. The emir and his village headmen denied the existence of sleeping sickness in their villages, but later, as the first cases were being diagnosed at the survey, they admitted knowing four villages where sickness was common and where the population was tending to decrease. These villages were later found roughly to correspond with three foci of moderate endemicity of trypanosomiasis.

The findings of the mass surveys were as follows :

District	No. examined	Cases	Percentage of infection
Dass	14,615	196	1·34
Toro	25,099	298	1·15

The number of cases of trypanosomiasis was pleasantly low. Many villages and hamlets, especially in the higher hills south and east of Toro, were completely free of the infection. The proportion of advanced toxic or somnolent cases was about the usual low average for similar surveys in Northern Nigeria.

The cases, the results of the treatment of which are here described, came from the villages of Bagel and Bakin Kogi in Dass District, and from Laro

village in Toro District. The infection-rates of these villages were respectively 10 per cent., 3 per cent. and 16 per cent.

TREATMENT WITH 4 : 4'-DIAMIDINO STILBENE

The experimental work leading to the discovery of the trypanocidal activity of diamidino stilbene and related drugs is described by Lourie and Yorke (1939) and by Yorke (1940). The drug was supplied in ampoules of 50 mgm. and 100 mgm. Solution, in cold distilled water, was effected by vigorous shaking for 5-10 minutes. The wide-mouthed stoppered 250 c.cm. bottles used in mass treatment with other drugs were found most useful for this purpose. One hundred mgm. of the drug can be dissolved in 10 c.cm. of water, and this was the strength of solution used. Injection was normally made intravenously, and at a slow rate, the injection being stopped if a severe reaction seemed imminent.

Groups of patients from two villages were treated.

From Bagel village, in Dass District, eight cases were chosen (Table I). All had marked enlargement of the cervical glands ; some a less marked enlargement of the axillary glands. Three denied symptoms ; a fourth admitted to pain in the neck-glands only ; but the remaining four gave a history of recurring attacks of headache or fever, or both, sometimes with backache. None showed wasting. Loss of strength was admitted by four, and two showed some facial puffiness. Tremors, lethargy and mental hebetude were not noted. Accepting the figures given by Greenfield and Carmichael (1925) as standards for the normal cerebrospinal fluid (i.e., a cell count of not more than 3 per c.mm., with either no opalescence or a faint haze to the Ross-Jones test with saturated ammonium sulphate), only one cerebrospinal fluid—that of case no. 80—was within normal limits. No specimen had a cell count greater than 10 per c.mm., and the increases in globulin were slight or moderate. The average duration of symptoms was eight months. These cases were, if anything, rather milder than those normally met with in Nigeria, even in mass-survey work.

The six patients from Laro village, in Toro District (see Table II), showed a more advanced condition. Glandular enlargements were less marked. All gave histories of recurrent attacks at frequent intervals, and all except one had lost strength. Lumbar puncture was permitted in three cases only. One man, who looked well, had a severe pleocytosis of 196 cells per c.mm., with globulin in slight excess. Two others, with a normal globulin reaction, had slight and moderate pleocytosis respectively. The average duration of symptoms for all cases was 16 months.

The Laro cases showed more reaction to the drug than the Bagel cases and therefore received lower doses, averaging 6·3 mgm. per kilo., as against the 8·8 mgm. per kilo. given to the Bagel group.

Examination of gland-juice during treatment showed that sterilization of the glands occurred after one injection in one case, after two injections in eleven

TABLE I

Results of treatment with diamidino stilbene of 8 patients from Bagel village, Dass. Treatment given on 1st, 3rd, 5th, 7th, 8th and 9th days. Injections usually intravenous; on 5th day intramuscular injections given to all cases except nos. 85 and 82. Individual doses from 0·7 to 2·2 mgm. per kilo.

Before treatment, trypanosomes present in gland-juice of all cases.
After treatment, no trypanosomes in gland-juice or blood.

Case no.	Before treatment			Total dosage: mgm. per kilo.	Interval since treatment	After treatment		
	Clinical condition	C.S.F.				Clinical condition	C.S.F.	
		Cells per c.mm.	Globulin				Cells per c.mm.	Globulin
85	F.14. Flabby build; face puffy. Duration 6 mths. Wgt. 39 kilo.	9	+	8·8	3 wks.	Improved. Wgt. 41 kilo.		
					6 mths.	Well; strong; no puffiness. Wgt. 42 kilo.	2	—
82	F.30. Slender; debility not marked. Duration 12 mths. Wgt. 48 kilo.	6	+	9·3	3 wks.	Stronger. Wgt. 48 kilo.		
					6 mths.	Well. Wgt. 52 kilo.	6	∓
86	M.12. Thin; not well; denies symptoms. Duration ? 6 mths. Wgt. 22 kilo.	2	++	9·0	3 wks.	Improved. Wgt. 24 kilo.		
					6 mths.	Well; still too thin. Wgt. 26 kilo.	2	—
102	F.36. Slender; debility slight. Duration 4 mths. Wgt. 49 kilo.	4	—	9·7	3 wks.	Improved. Wgt. 50 kilo.		
					6 mths.	Well. Wgt. 50 kilo.	Dry tap	
78	M.16. Strong; few symptoms; face puffy. Duration 14 mths. Wgt. 40 kilo.	7	+	7·4	3 wks.	Improved. Wgt. 41 kilo.		
					6 mths.	Well; no puffiness	2	∓
80	M.10. Well built; said not to be strong. Duration ? 1 yr. Wgt. 28 kilo.	2	∓	10·4	3 wks.	Wgt. 29 kilo.		
					6 mths.	Well; strong. Wgt. 31 kilo.	Blood	
81	M.11. Thin; nervous; unwell; denies symptoms. Duration ? 9 mths. Wgt. 24 kilo.	2	+	9·4	3 wks.	Improved. Wgt. 26 kilo.		
					6 mths.	Well; sturdier. Wgt. 29 kilo.	1	—
88	F.38. Thin; weak; nervous. Duration ? Wgt. 45 kilo.	6	++	6·6	3 wks.	Improved. Wgt. 45 kilo.		
						Died following abortion 4 mths. after treatment		

ROSS-JONES GLOBULIN TEST

— denotes normal (no opalescence).
∓ ,, probably normal (faint haze).
\+ ,, slight excess of globulin (faint ring).
++ ,, moderate excess of globulin (heavy ring).
+++ ,, marked excess of globulin (very heavy ring).

cases, and after three injections in two cases. The dosage required to produce this effect varied from 0·9 to 5·0 mgm. per kilo.

The Bagel cases, who had less severe symptoms and who appeared fairly well before treatment, said that they felt better at the end of the treatment.

TABLE II

Results of treatment with diamidino stilbene of 6 patients from Laro village, Toro. Treatment given on 1st, 2nd, 5th, 6th, 7th and 8th days. Injections all intravenous. Individual doses from 0·5 to 1·5 mgm. per kilo.

Before treatment, trypanosomes present in gland-juice of all cases.
After treatment, no trypanosomes in gland-juice. Blood not examined.

Case no.	Before treatment					After treatment		
		C.S.F.					C.S.F.	
	Clinical condition	Cells per c.mm.	Globulin	Total dosage: mgm. per kilo.	Interval since treatment	Clinical condition	Cells per c.mm.	Globulin
18	M.30. Symptoms severe, but looks well. Duration 2 yrs. Wgt. 60 kilo.	196	+	6·6		Died 9 days after treatment		
24	M.38. Thin; looks ill; frequent short attacks. Duration 18 mths. Wgt. 60 kilo.	17	—	5·5	6 mths.	No symptoms, but does not look well. Wgt. 60 kilo.	6	—
54	M.22. Thin; looks ill; short attacks at very frequent intervals. Duration 12 mths. Wgt. 51 kilo.	9	—	6·9	6 mths.	No symptoms; still looks ill. Wgt. 48 kilo.	5	—
33	F.50. Looks fairly well; fever almost daily. Duration 10 mths. Wgt. 43 kilo.			6·8	6 mths.	No fevers; general appearance not altered. Wgt. 44 kilo.		
30	F.42. Obese; looks well. Duration 2 yrs. Wgt. 59 kilo.			6·0	6 mths.	No symptoms; looks well. Wgt. 65 kilo.		
35	F.6. Slender; looks fairly well. Duration 8 mths. Wgt. 20 kilo.			6·2	6 mths.	No symptoms; looks better; still rather slender. Wgt. 22 kilo.		

When seen three weeks later, they were livelier, less easily fatigued, had gained weight, and their glands were smaller and firmer. Gland punctures showed no trypanosomes.

The Laro group improved in appearance during treatment. From being quiet and rather worried, they became more cheerful and confident regarding

treatment. In those who had previously had almost constant symptoms, headache and fever disappeared. All said that they felt better and stronger.

After three months, gland punctures in the 13 patients still alive were kindly done for me by Dr. C. H. Smith, of Bauchi, and by the Toro dispensary attendant. No trypanosomes were seen.

The last re-examinations were made a little more than six months after treatment. One Bagel patient had died following an abortion, but was said to have been in good health previously. The other seven had maintained improvement. They looked, and said that they felt, well. Six showed further gain in weight. Palpable glands were fewer and smaller, and were firm to stony-hard in consistency. No trypanosomes were seen in gland-juice or in blood. Of the five cerebrospinal fluid specimens obtained, four, which had previously shown slight abnormalities either in cell count or in globulin content, were within normal limits. The fifth had the same cell count of 6 as before, but the globulin content, previously slightly increased, was now normal.

One of the Laro patients died soon after treatment. His symptoms resembled those of tetanus, or may have been due to poisoning. Friends stated that they recognized the disease, and that his death was unconnected with his previous illness. Although the remaining five cases insisted that symptoms had not returned, only one (no. 30) looked really well, and one other (no. 35) looked better than before treatment. Both had gained weight. The others appeared much as before treatment, despite the apparent absence of symptoms, and one had lost weight. Glands had decreased in number and in size, but in case no. 24 were still moderately enlarged and fairly soft. No trypanosomes were seen in gland-juice. The blood-films taken were destroyed accidentally. In the two specimens of cerebrospinal fluid examined, the cell counts had fallen, but were not normal. There was still no excess of globulin in either.

Toxic Effect of Diamidino Stilbene

So far as was observed, the drug caused no late toxic reaction, and patients, after resting for two hours after treatment, were able to return home. As is customary in mass treatment with Bayer 205 or tryparsamide, advice was given that, on the morning of treatment, no food, or only the lightest of food, should be taken, and that early in the morning, at least two hours before treatment.

Toxic action was noted during and immediately after the intravenous injection of the drug. The cases from Dass showed little reaction ; those from Toro had more constant and more severe reactions.

Dass Cases

After intravenous injection, slight giddiness was almost always present. After treatment, the patients sat or lay semi-recumbent, were given cold water to drink if requested, and were not worried by the giddiness, which passed off in $\frac{1}{2}$–2 minutes. The initial dose of approximately 1 mgm. per kilo. caused

practically no upset—so little, in fact, that at the time it was neither admitted by the patients nor evident to the observer. In one case, this initial dose caused slight salivation.

In three patients the second injection caused a slightly more marked reaction —salivation, nausea, giddiness or faintness. Five patients, given from 1·5 to 2 mgm. per kilo., had no reaction, or had giddiness only.

Following this second injection, four patients complained of fever and discomfort after walking home, but these were the only cases in which such a complaint was heard.

Immediately after the third treatment, the first two patients, given a dose similar to, or less than, that previously borne without upset, were immediately distressed, feeling dizzy and faint. Both were comfortable again in a few minutes, and were normal in less than two hours. The other patients therefore received their injection intramuscularly, with no upset at the time. It was considered at the time that the reaction to intravenous injection was due to imperfect solution of the drug. The last three injections, of from approximately 1 to 2 mgm. per kilo., caused the usual slight giddiness only.

Intramuscular injection given to six patients on the 5th day caused considerable local pain during the following night.

Toro Cases

The Toro cases showed considerable reaction to the drug, and the doses had to be chosen arbitrarily according to the individual reaction during injection. The highest dose achieved was 1·5 mgm. per kilo., and the total for the course varied from 5·4 mgm. per kilo. to 6·9 mgm. per kilo. Neither the cerebrospinal fluid findings nor the appearance of the patient clinically gave any indication of the reaction to be expected.

There was an immediate reaction to every injection—precordial distress, dizziness, tingling in the extremities, salivation, some headache—which never lasted longer than 20 minutes. After resting for one to two hours, the patients could walk three miles home without upset. During the course some tolerance seemed to be acquired, the reaction beginning a little later (though always during the injection) and not lasting so long.

Albuminuria

The eight Dass patients had the urine tested for albumin and sugar before treatment and on each of the six days on which they attended for treatment. None had glycosuria at any time, and, before treatment, none had albuminuria. During treatment, four remained free from albuminuria. No. 82 had a trace of albumin on the 6th and 8th days ; none on the 9th day. No. 86 had a trace on the 9th day—the last day of treatment. No. 102 had albuminuria on the 5th day, but none on succeeding days. (Nos. 82 and 102 are women.) Such

transient albuminuria, even if due to the drug, can be neglected, though Adler and Tchernomoretz (1939) describe renal damage in the hamster after a single very large dose.

TABLE III

Results of treatment with Bayer 205 of 9 patients from Bakin Kogi village, Dass. Six injections of Bayer 205 at 5-day intervals. Dosage for an adult male : a preliminary injection of 0·2 gm., then 5 of 1 gm.
Before treatment, trypanosomes present in gland-juice of all cases.
After treatment, no trypanosomes seen in gland-juice. Patients absconded before blood-films taken.

Case no.	Before treatment					After treatment		
		C.S.F.					C.S.F.	
	Clinical condition	Cells per c.mm.	Globulin	Dose of Bayer 205 : gm.	Interval since treatment	Clinical condition	Cells per c.mm.	Globulin
175	M.12. Well-nourished ; face puffy. Duration 14 mths. Wgt. 31 kilo.	4	—	3·15	7 mths.	No symptoms ; no puffiness. Wgt. 32 kilo.	10	∓
177	M.12. Slender ; some debility. Duration 4 yrs. Wgt. 28 kilo.	14	+	3·15	7 mths.	No symptoms ; stronger. Wgt. 31 kilo.	3	+
178	M.12. Face puffy ; some debility. Duration 2 yrs. (? 5 yrs.) Wgt. 32 kilo.	Blood		3·15	7 mths.	Very well ; no symptons or puffiness. Wgt. 34 kilo.	6	∓
179	F.16. Slim ; some debility ; looks well. Duration 5 yrs. Wgt. 49 kilo.	3	—	5·2	7 mths.	No symptoms ; 3 mths. pregnant. Wgt. 47 kilo.	2	+
182	M.15. Looks fairly well ; some debility. Duration 3 yrs. Wgt. 33 kilo.	11	∓	3·15	7 mths.	No symptoms. Wgt. 35 kilo.	14	—
183	F.45. Poor physique ; facile ; denies symptoms. Duration ? Wgt. 43 kilo.	10	—	3·9	7 mths.	Not improved. Wgt. 41 kilo.	4	∓
187	M.40. Dull ; symptoms fairly severe. Duration 3 yrs. Wgt. 63 kilo.	Blood		5·2	7 mths.	Well ; less dull. Wgt. 64 kilo.	Blood	
198	M.13. Slender ; face puffy ; symptoms severe. Duration 1 yr. Wgt. 35 kilo.	11	∓	3·15	7 mths.	No puffiness ; no symptoms. Wgt. 39 kilo.	6	∓
199	M.45. Thin ; slow mentally. Duration 10 mths. Wgt. 51 kilo.	14	+	4·2	7 mths.	No symptoms. Wgt. 52 kilo.	Blood	

TREATMENT WITH BAYER 205

From Bakin Kogi village, in Dass District, 18 cases were treated with Bayer 205 alone (see Table III). Nine who were re-examined form a group fairly comparable with those treated with diamidino stilbene. Their symptoms

TABLE IV

Results of treatment with Bayer 205 and tryparsamide of 7 cases from Bagel village, Dass. Ten injections at intervals of 5 days. Dosage for an adult male : a preliminary injection of Bayer 205 0·2 gm., followed by Bayer 205 3 injections of 1 gm. and tryparsamide 5 injections of 2 gm.

Before treatment, trypanosomes present in gland-juice of all cases.
After treatment, no trypanosomes in gland-juice or blood.

Case no.	Before treatment					After treatment		
		C.S.F.					C.S.F.	
	Clinical condition	Cells per c.mm.	Globulin	Total dosage : in gm.	Interval since treatment	Clinical condition	Cells per c.mm.	Globulin
79	M.16. Slender; not strong ; denies symptoms. Duration ? Wgt. 38 kilo.	9	—	B. 2·45 T. 7·9	5 mths.	Well; strong; no symptoms. Wgt. 43 kilo.	4	∓
84	F.26. Slender; fairly well; symptoms mild. Duration 1 yr. Wgt. 45 kilo.	1	+	B. 2·6 T. 7·9	5 mths.	Well ; no symptoms ; 3 mths. pregnant. Wgt. 55 kilo.	5	+
87	F.35. Symptoms denied. Duration ? Wgt. 47 kilo.	8	++	B. 3·2 T. 10·0	5 mths.	Well ; 5 mths. pregnant. Wgt. 55 kilo.	No lumbar puncture	
90	M.18. Strong ; symptoms slight. Duration 1 yr. (? 5 yrs.). Wgt. 52 kilo.	16	—	B. 3·2 T. 10·0	5 mths.	No symptoms. Wgt. 55 kilo.	6	+
91	M.9. Slender; fairly severe symptoms. Duration 8 mths. Wgt. 22 kilo.	2	+	B. 1·3 T. 4·0	5 mths.	No symptoms ; sturdier. Wgt. 27 kilo.	2·5	+
92	F.14. Slim ; poorly developed. Duration 6 mths. (? 5 yrs.). Wgt. 33 kilo.	4	∓	B. 1·9 T. 6·0	5 mths.	No symptoms. Wgt. 40 kilo.	2·5	+
104	F.31. Well-nourished ; amenorrhoea ; symptoms slight. Duration 6 mths. (? longer). Wgt. 64 kilo.	12	+	B. 3·2 T. 10·0	5 mths.	No symptoms ; ? 2 mths. pregnant. Wgt. 71 kilo.	7·5	+

Case no. 91 is brother of case no. 86 and son of case no. 102, both in Table I. The father died of trypanosomiasis before treatment began.

were intermediate in severity between those of the Bagel and those of the Laro cases. Although the average duration of the symptoms was much longer (about 30 months), their general condition was remarkably good. Seven had lumbar

puncture performed before treatment. One has a cerebrospinal fluid within normal limits. Six had counts ranging from 4 to 14 cells per c.mm., and two of these showed slight excess of globulin.

Seven months after treatment, all said that they had been well since treatment. Case no. 183, who had probably had a long-standing infection, showed no improvement clinically. Two cases appeared well, but in the other six cases the impression gained was that, though they had improved and appeared to be free from symptoms, there was room for further improvement. None of the seven lumbar punctures performed gave a completely normal fluid. The cell count had increased in two cases and was reduced in three, and the globulin showed excess in one case over the findings before treatment, though all such changes were slight. Glandular enlargements were smaller and fewer, the remaining glands being firm or stony-hard. Gland puncture showed no trypanosomes.

TREATMENT WITH BAYER 205 AND TRYPARSAMIDE

The standard course of treatment with Bayer 205 and tryparsamide which is used in mass-survey work was given to seven patients (see Table IV). These patients are comparable with the eight from the same village treated with diamidino stilbene (Table I). Symptoms were not severe, nutrition was fair, and the average duration of symptoms was about seven months. Changes in the cerebrospinal fluid were slight, though no specimen was strictly normal in both cell count and globulin content. Five months after treatment all patients said that they had become free of symptoms. They showed more improvement than any other group. Palpable glands were few, small and fibrotic. No trypanosomes were seen in gland-juice or in blood-films. Two of the adult women were pregnant, and a third, who had had amenorrhoea previously, now thought that she had an early pregnancy. Again, in none of the six specimens obtained was the cerebrospinal fluid quite normal: one showed an increase in cells, and two showed an increase in globulin not previously present.

SUMMARY

The findings of mass surveys for trypanosomiasis in two previously unexamined districts of the Bauchi Province of Northern Nigeria are given. Infection-rates were low.

Fourteen cases of sleeping sickness were treated with diamidino stilbene. The first group of eight cases were of a mild nature: they received an average of 8·8 mgm. per kilo. of the drug; after six months, seven were in good health; one had died following an abortion. The second group of six showed a more advanced condition: they showed reaction to intravenous injection of the drug and received an average of only 6·3 mgm. per kilo.; they appeared to improve during treatment; one died nine days after treatment; after six months, only two were really well, though symptoms appeared to be absent in all five.

Nine cases of a severity intermediate between the first two groups had Bayer 205 alone. After seven months, symptoms were in abeyance, but improvement in general condition was not marked.

Seven mild cases from the same village as the first group had Bayer 205 and tryparsamide. After five months they were well, showing more improvement in general condition than did any of the other groups.

Trypanosomes disappeared from the gland-juice after one to three injections of diamidino stilbene. Sclerosis of glands was much the same in all groups, and gland-juice was sterile at the last re-examination in all cases.

Cases were too few, and the original changes in the cerebrospinal fluid too slight, for alterations as the result of treatment to be of much significance. After treatment with diamidino stilbene, no case showed increase in the cell count or in the globulin content of the cerebrospinal fluid beyond that found initially, and four fluids, previously abnormal in some respect, had become normal. With the other drugs, slight increases either in cell count or in globulin content followed treatment in some cases, and no specimen became strictly normal.

The toxic effect of the drug is described. This is slight. Reaction occurs during intravenous injection, usually decreases in a few minutes and does not last more than 20 minutes. Care is required in the preparation and injection of the solution. The dosage given is subject to the reaction produced in the individual, but most patients can stand from 1 mgm. to 1·5 mgm. per kilo., and some 2 mgm. per kilo.

In cases with no marked involvement of the nervous system, diamidino stilbene appears to be as effective as Bayer 205, but less effective than the combination of Bayer 205 and tryparsamide, in the doses described. The shorter course, eight to nine days, or even less, is a great advantage, but courses of, say, ten injections are probably advisable for routine treatment.

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NOTES ON SOME CASES OF SUDAN KALA-AZAR TREATED WITH 4 : 4′-DIAMIDINO STILBENE

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INTRODUCTION

In August, 1939, Professor Warrington Yorke kindly forwarded to us samples of 4 : 4′-diamidino stilbene (see Lourie and Yorke, 1939), with the suggestion that we should try this drug in human cases of kala-azar. It had already been shown that this compound could cure hamsters infected with *Leishmania donovani* (Adler and Tchernomoretz, 1939), while a very satisfactory result had been obtained in a case of Indian kala-azar treated in Liverpool (Adams and Yorke, 1939) and in a case of Mediterranean kala-azar (Adler and Rachmilewitz, 1939). As the results of treatment in Sudan kala-azar are at present unsatisfactory, we were very glad to try the new compound, and during the past eight months have used it in 28 cases in Singa hospital. Chronologically these fall into two separate groups. The first consists of eight cases which were treated during the latter part of 1939, and have been kept under observation since their discharge from hospital at the beginning of 1940. The second group comprises 20 cases, some of which have had severe complications, such as cancrum oris and pneumonia. Of this group, two have died, three are still in hospital, and the remainder have been discharged recently as apparent cures. These cases are being followed up, and an account of them will be published later.

CASE-HISTORIES

In the summaries which follow, irrelevant negative findings are omitted. In all cases the urine and stools have been examined for parasites and ova; where fever occurred the blood was repeatedly searched for malaria, and in all cases has been tested for agglutinins to typhoid, paratyphoid and Malta fever. Except where the contrary is stated, it may be taken that these examinations have been negative. Most of the patients complained on admission of fever varying in duration from one to four months, but no reliance can be placed on those histories and, for the sake of brevity, they have been omitted. The degree of hepatic and splenic enlargement was estimated at regular intervals by measuring the number of fingers' breadth to which the organs extended below the costal margin; this is recorded briefly as so many F.B. In all cases nasal smears have been examined for Leishmania, with negative result.

Cultural methods are hardly feasible in Sudan out-stations, and to determine the presence or absence of Leishmania parasites reliance has been placed on the microscopical examination of stained smears of material obtained by splenic puncture and gland puncture, the latter being carried out by the method recently described by the present writers (1940*a*). With regard to the differential blood count, it may be stated that some increase in the lymphocytes at the expense of the polymorphs may be regarded as a fairly normal finding in this country, both in Sudanese and in Europeans who have spent several years in the Sudan.

CASE NO. 1

Male, aged 10 years, Bernawi tribe, admitted on August 30th, 1939, as a medico-legal case, after having been attacked and beaten. On admission, was found to have fever ranging to 104° F. General condition very weak and drowsy. Oedema of face and legs, tympanites, ulcerations on the inside of the lips. Spleen 3 F.B. ; liver just palpable on inspiration. Blood : R.B.C. 1,485,000, W.B.C. 2,200. Blood-film contained B.T. malaria, for which appropriate treatment was instituted. This had no effect on the fever, and on September 14th Leishmania were found in the splenic pulp, gland-juice, and in a skin-scraping from the face.

On September 23rd, 25th and 27th, he was given 25 mgm. of 4 : 4′-diamidino stilbene intravenously (1 mgm. per kilo. body weight). On the 27th the temperature fell, and the patient died on the following afternoon, having had a total of 3 mgm. per kilo. of the drug over a period of 5 days.

CASE NO. 2

Female, aged 9 years, Gablawia tribe, admitted on September 7th, 1939. General condition on admission weak. Fever ranging to 104° F. Weight 3 stone. Spleen 3 F.B. ; liver 1 F.B. Blood : R.B.C. 2,920,000, W.B.C. 2,400 ; differential count : P. 32 per cent. ; L. 58 per cent. ; L.M. 10 per cent. ; E. 0. The urine contained albumin, bile, red blood corpuscles, granular casts and oxalate crystals. Spleen puncture on September 20th revealed Leishmania parasites.

From September 23rd to October 11th, the patient was given 10 injections of 25 mgm. (1·3 mgm. per kilo. body weight) of 4 : 4′-diamidino stilbene, on alternate days. During this course the temperature subsided. On October 10th the patient developed a sharp attack of diarrhoea ; blood, mucus and flagellates (*Chilomastix*) were found in the stool. On October 12th, splenic pulp and gland-juice were still positive for Leishmania. The temperature began to rise again, diarrhoea recurred, and the patient lost weight.

From October 25th to November 6th, a second course of 7 injections of 25 mgm. was given on alternate days, and the fever again subsided.

On November 15th it was found that the splenic pulp was still positive for Leishmania, so a third course of 10 injections was given on alternate days from November 17th to December 5th. For the last six of these the dose was increased to 50 mgm. (2·6 mgm. per kilo.). There was some rise of temperature (to 102° F.) during this course, but by the end of the course it had settled. On December 7th the condition of the blood was R.B.C. 3,310,000, W.B.C. 4,200 ; differential count : P. 45 per cent. ; L. 51 per cent. ; L.M. 1 per cent. ; E. 3 per cent. The general condition was very much better, and a fine punctate rash had appeared on the face, but no Leishmania were found in skin-scrapings.

Spleen puncture, however, was positive on December 13th and on December 19th. So a fourth course of 8 daily injections of 50 mgm. was given from December 26th to January 2nd, 1940, during which there was no recurrence of fever. Spleen and gland puncture were negative on January 9th. On January 10th, diarrhoea recurred but cleared up rapidly under treatment. During the next 10 days the spleen shrank rapidly, and by January 20th was not palpable, while the red blood count had risen to 4,650,000, and the white count to 5,200.

The patient, who had gained 13 lb. since admission, left hospital on this date, but was seen later on April 26th in her village. There had been no recurrence of fever. Nutrition and general condition were excellent, the spleen was not palpable, and the punctate rash on the face was beginning to diminish.

CASE No. 3

Male, aged 23 years, Bernawi tribe, admitted on September 9th, 1939. General condition fair. Mildly delirious on admission, and temperature 105° F. Weight 7 stone. Spleen 3 F.B.; liver 4 F.B. and hard. Blood: R.B.C. 2,110,000, W.B.C. 4,000; differential count: P. 49 per cent.; L. 48 per cent.; L.M. 3 per cent.; E. 0. The urine contained albumin and pus. A blood-film revealed the parasites of M.T. malaria, for which appropriate treatment was instituted. On September 14th splenic pulp, gland-juice and bone-marrow were all found to contain Leishmania, which were also found in a scraping from a small sore on the face.

From September 23rd to October 2nd a course was given of 10 daily intravenous injections of 50 mgm. (1·1 mgm. per kilo. body weight) of 4 : 4′-diamidino stilbene. The temperature subsided three days after the completion of this course, but spleen and glands still contained Leishmania.

Ten days later the temperature again began to rise, and a second course of 8 daily injections of 50 mgm. was given from October 23rd to the 30th. The first few injections caused some exacerbation of the fever, but this settled before the completion of the course.

On November 7th spleen and glands were still positive for Leishmania, and a third course of 8 daily injections of 100 mgm. was given from November 16th to the 23rd. For the following four weeks there was every day a slight evening rise of temperature to 99° F., and spleen and glands remained positive. Meantime, there was some improvement in the blood count, which was as follows on December 3rd: R.B.C. 4,050,000, W.B.C. 6,000; differential count: P. 34 per cent.; L. 57 per cent.; L.M. 6 per cent.; E. 3 per cent.

On December 23rd the temperature again began to rise, and a fourth course of 10 daily injections of 100 mgm. was given, during which there was fever rising to 103° F., and a severe epistaxis, and the patient lost 9 lb. in weight. The spleen enlarged to 5 F.B. and a nodular rash appeared on the face. On the completion of this course the temperature became normal. Spleen puncture on January 9th, 1940, was negative, and the splenic tumour began to shrink. Spleen and glands were negative for Leishmania on January 16th, and four days later the spleen was not palpable. The blood count on the 16th was: R.B.C. 4,660,000, W.B.C. 4,000; differential count: P. 57 per cent.; L. 39 per cent.; L.M. 2 per cent.; E. 2 per cent. Urine contained only a trace of albumin. The eruption on the face had by this time become more prominent, but repeated scrapings failed to reveal Leishmania even from the site where they had been found previously.

The patient was discharged on January 21st, having gained 12 lb. in weight since admission. There has been no relapse in the ensuing four months, during which period the patient has been back at his work. The skin eruption is still present and the liver is palpable on inspiration. A blood count on February 30th showed a moderate leucocytosis of 9,800.

CASE No. 4

Female, aged 9 years, Fur tribe, admitted on September 26th, 1939. General condition very weak, almost moribund. Fever ranging between 104° and 105° F. Spleen 3 F.B.; liver 2 F.B. The urine contained albumin, pus and epithelial cells. Leishmania were found in the gland-juice. On account of the grave general state of the patient, no further examinations were made, except to exclude the presence of malaria.

Treatment was started with 1 injection of neostibosan (0·2 gm.) on September 27th. Owing to the death of case no. 1 on September 28th, the remainder of his course of 4 : 4′-diamidino stilbene became available for this patient, who was given 5 injections of 25 mgm. (1·3 mgm. per kilo.) on alternate days from September 29th to October 7th. On October 7th, there was marked puffiness of the face, and generalized oedema. The urine was loaded with albumin, and contained hyaline and granular casts as well as epithelial

cells. The patient died the same night, having had a total dosage of 125 mgm. distributed over a period of 9 days.

CASE NO. 5

Male, aged 35 years, Habani tribe, admitted on September 28th, 1939. General condition fair. Weight 6 stone, 12 lb. Fever rising to 102° F. The spleen was enlarged to the umbilicus, and the liver was palpable on inspiration. Blood : R.B.C. 2,750,000, W.B.C. 3,000 ; differential count : P. 56 per cent. ; L. 43 per cent. ; L.M. 1 per cent. ; E. 0. The urine contained only a trace of albumin and a few pus cells. Splenic pulp and gland-juice contained Leishmania.

From September 29th to October 8th the patient was given 10 daily intravenous injections of 50 mgm. 4 : 4′-diamidino stilbene (1·1 mgm. per kilo. body weight). During this course the temperature subsided completely by lysis, and a remarkable improvement took place in the general condition and appearance of the patient. On October 10th splenic and gland punctures were still positive, and the blood picture and the size of the spleen were unchanged. Fourteen days later, on October 24th, sternal puncture and gland puncture were both negative.

A second course of 8 daily injections of 50 mgm. was given between October 23rd and the 30th. These had no effect on the temperature : the patient remained afebrile. On November 6th, however, gland puncture was positive, as was spleen puncture on the 7th, but the blood count had improved very considerably—R.B.C. 4,220,000, W.B.C. 5,000 ; differential count : P. 53 per cent. ; L. 43 per cent. ; L.M. 4 per cent. ; E. 0.

Severe epistaxis, recurring almost daily, began on November 12th. The temperature, which had remained normal since the completion of the first course, began to rise again, so a third course of 8 daily injections (100 mgm. alternating with 75 mgm.) was given between November 16th and the 23rd. During this course, there were several severe epistaxes, fever rising to 103° F., the splenic enlargement increased, and the patient lost 11 lb. in weight. On completion of the course, the temperature fell to normal. Splenic puncture was negative on December 2nd, and again on the 13th. The weight increased rapidly, and the spleen began to shrink, until on the 22nd it was only 2 F.B. On January 17th, 1940, the blood count was as follows : R.B.C. 5,110,000, W.B.C. 5,500 ; differential count : P. 50 per cent. ; L. 43 per cent. ; L.M. 3 per cent. ; E. 4 per cent.

The patient was discharged as cured on January 18th, having gained 26 lb. in weight since admission. He resumed his employment and remained well during the next four months. On March 30th the blood picture was similar, except for a moderate leucocytosis of 9,200. The spleen is still palpable 2 F.B.

CASE NO. 6

Male, aged 20 years, of Tamawi tribe, admitted on October 22nd, 1939. General condition fair, temperature 104° F. Weight 6 stone. Spleen 5 F.B. ; liver 2 F.B. Urine contained albumin, pus cells and granular casts. Blood : R.B.C. 2,750,000, W.B.C. 4,000 ; differential count : P. 50 per cent. ; L. 44 per cent. ; L.M. 6 per cent. ; E. 0. Spleen-smears positive for Leishmania.

From November 14th to November 23rd he was given a course of 10 daily injections of 50 mgm (1·2 mgm. per kilo.) of 4 : 4′-diamidino stilbene. This appeared to cause some exacerbation of the fever, and there were several troublesome epistaxes, but the high fever came down on November 26th. On December 1st spleen-smears were positive for Leishmania, and again on December 7th both spleen- and gland-smears were positive. The blood picture remained unchanged, except for a slight rise in lymphocytes.

From December 12th to the 19th another course of 8 daily injections of 100 mgm (2·4 mgm. per kilo.) was given, which brought on a sharp exacerbation of fever for the first five days, after which the temperature subsided and remained normal. On December 24th it was noticed that the spleen had begun to shrink. Spleen-smear on December 26th and gland-smear on the 30th were both negative. There was still little change on the blood picture. By January 20th, 1940, spleen and liver had shrunk to 1 F.B. Except for the access of fever during the second course, the patient had been afebrile for 8 weeks.

As he was becoming restive in hospital, he was given a further course of 8 daily injections of 100 mgm., and was then discharged on January 21st. During this course he developed a punctate rash on the face.

He had gained 9 lb. in weight during his stay in hospital. When seen on February 29th, after he had been back at his work for a month, his general condition was excellent, with no return of fever. Spleen and liver still 1 F.B. Blood : R.B.C. 3,440,000, W.B.C. 5,800 ; differential count : P. 51 per cent. ; L. 42 per cent. ; L.M. 5 per cent. ; E. 2 per cent. The punctate rash was still present, and on his neck was a small nodular dermal leishmanoid similar to that described by Balfour and Thomson (1911), and skin-scrapings of this were positive for Leishmania.

On May 17th, when last seen, his condition was unchanged ; blood picture was : R.B.C. 4,340,000, W.B.C. 5,400 ; differential count : P. 41 per cent. ; L. 50 per cent. ; L.M. 6 per cent. ; E. 3 per cent.

CASE NO. 7

Male, aged 28, Abu Rof tribe, admitted on October 25th, 1939. High fever (104° F.) on admission, but general condition otherwise fair. Weight 8 stone, 1 lb. Spleen 3 F.B. ; liver 2 F.B. and hard. Blood : R.B.C. 2,850,000, W.B.C. 2,200 ; differential count : P. 50 per cent. ; L. 48 per cent. ; L.M. 2 per cent. ; E. 0. Urine contained albumin, pus, and a few red blood cells. Spleen- and gland-smears positive for Leishmania.

Beginning on October 25th, the patient was given a series of 13 daily intravenous injections of 4 : 4′-diamidino stilbene. The dosage was 50 mgm. (1 mgm. per kilo. body weight) every second day, and 25 mgm. on the days intervening. (This peculiar system of dosage was adopted purely as a matter of convenience, to distribute the available drug among several patients without wastage.) After completion of this course, spleen- and gland-smears were found to be still positive for Leishmania, and the temperature showed little tendency to subside.

From November 16th to the 25th, a second course of 10 daily injections of 50 mgm. was given. During this course there was an exacerbation of fever, which suddenly ceased when the course was completed, but the spleen had increased in size to 5 F.B. The patient was kept in hospital for a further seven weeks without treatment, during which the spleen shrank again to 2 F.B. The temperature remained normal, and spleen- and gland-smears were negative for Leishmania. There was no change in the liver. Urine contained only a trace of albumin and a few pus cells.

On January 17th, 1940, blood showed : R.B.C. 3,900,000, W.B.C. 2,600 ; differential count : P. 48 per cent. ; L. 42 per cent. ; L.M. 5 per cent. ; E. 5 per cent. A papular eruption had appeared on the face, but scrapings made from the papules failed to reveal Leishmania. The patient refused to stay in hospital any longer, and was discharged on January 20th. He had gained 7 lb. in weight since admission.

He was seen three months later. No recurrence of fever. General condition excellent. No change in liver and spleen. Blood : R.B.C. 4,260,000, W.B.C. 5,200 ; differential count : P. 42 per cent. ; L. 52 per cent. ; L.M. 2 per cent. ; E. 4 per cent. The papular rash was still present, but less prominent.

He was seen again at the end of May, 1940, six months after the last injection of the drug. Liver and spleen had both become non-palpable. The blood count remained the same, except that the R.B.C. had risen to 4,810,000. The rash appeared to be fading.

CASE NO. 8

Male, aged 30 years, Mahas tribe, admitted on November 7th, 1939. General condition fair. Weight 7 stone. Fever ranging from 100° F. to 104° F. Spleen 6 F.B. ; liver 3 F.B. Blood : R.B.C. 3,510,000, W.B.C. 2,200 ; differential count : P. 53 per cent. ; L. 43 per cent. ; L.M. 3 per cent. ; E. 1 per cent. Urine contained albumin, hyaline casts and pus cells. Spleen-smear and gland-juice both positive for Leishmania.

On November 14th the patient was given an injection of 60 mgm. of 4 : 4′-diamidino stilbene, followed by 7 daily injections of 100 mgm. (2·2 mgm. per kilo. body weight), the last on November 21st. After this the temperature did not settle completely owing to

the development of an abscess of the cheek, which discharged on December 7th. In the meantime glands had become negative for Leishmania, but parasites were found in the splenic pulp on November 29th and again on December 7th. The blood count and the size of the spleen and liver were unchanged.

Another course of 8 daily injections of 100 mgm. was given between December 12th and the 19th, during which there was some exacerbation of the fever. The temperature subsided completely, however, on the 20th, after which the patient remained afebrile and the spleen began to shrink. Gland puncture was negative for Leishmania on December 30th, and splenic puncture on December 27th, January 2nd and January 14th. Blood (January 17th): R.B.C. 3,850,000, W.B.C. 4,600; differential count: P. 57 per cent.; L. 40 per cent.; L.M. 1 per cent.; E. 2 per cent.

By January 20th the spleen had shrunk to 2 F.B. Urine contained a trace of albumin and a few pus cells. The patient had been afebrile for three weeks and had gained 19 lb. in weight, so he was given a further course of 8 daily injections of 100 mgm. and discharged from hospital.

He has remained well ever since and able to carry out all his duties, including a strenuous trek through the game-reserve, in search of poachers, and a lion-hunt. There has been no return of fever, and the blood picture has steadily improved. On May 18th the count was: R.B.C. 4,960,000, W.B.C. 6,000; differential count: P. 58 per cent.; L. 34 per cent.; L.M. 5 per cent.; E. 3 per cent. The spleen is still palpable on inspiration (1 F.B.).

EFFECTS OF 4:4′-DIAMIDINO STILBENE

The first effect of the drug is to reduce the fever and subjective symptoms of the disease. It may also produce a remarkable improvement in the general condition and well-being of the patient. Most of the patients consider themselves cured at this stage, and want to leave hospital. But the fever is liable to recur, and as a general rule considerably longer treatment is required to produce the desired improvement in the blood picture, negative parasitological findings, and reduction of the splenic tumour. The latter may undergo a noticeable enlargement during the course of treatment, and minor temporary fluctuations in size are common. There may be some discrepancy between the rate of improvement of the blood picture and the apparent disappearance of the parasites. In case no. 7 the improvement in the blood was not evident until some time after discharge from hospital, although parasites were persistently absent from the splenic pulp. On the other hand, in some of the later cases a rapid and more complete return of the blood picture to normal has been observed, in spite of the persistence of parasites in the splenic pulp.

Some interest attaches to the development of punctate and nodular eruptions during treatment. These closely resemble the skin rashes which often appear in cases of Sudan kala-azar during treatment with antimony. An account of these has been given elsewhere (Kirk and Sati, 1940*b*). It has been suggested that the primary cause of such skin eruptions is the leishmanial infection (it may be noted that parasites were found in the nodular eruption in case no. 6 in the present series), but the mechanism of their production is obscure. Experience suggests that the development of such eruptions during treatment has a good prognostic significance as far as the cure of the visceral condition is concerned. In this respect they bear some analogy to the post-kala-azar

dermal leishmaniasis of India, which appears after the clinical cure of the visceral condition and does not appear to be associated with relapse of the latter.

TOXICITY AND DOSAGE

In all instances the drug has been given by the intravenous route. One hundred mgm. dissolves with some difficulty in 10 c.cm. distilled water, and this was the concentration used for injection. Toxic symptoms have been negligible with the dosages employed, which varied from 1 to 2·6 mgm. per kilo. body weight. An exacerbation of fever is common when a new course of injections is started, even though the temperature has been normal for some time previously. Three of the early cases complained of transient epigastric discomfort, with breathlessness and dizziness after the first two injections, but these symptoms were not experienced with subsequent injections. In view of these reactions, we established a practice of giving the injections very slowly, and it may be for this reason that no further trouble was encountered in later patients. Two children developed epigastric pain and vomiting when the dosage was increased from 1·2 mgm. per kilo. on alternate days to 2·4 mgm. per kilo. daily; but these symptoms subsided rapidly when the dosage was reduced to 2·4 mgm. per kilo. on alternate days, and it was possible later to resume daily injections of this dosage without any symptoms supervening. The diarrhoea which occurred in case no. 2 is regarded as accidental. Several other patients in hospital at the time suffered from the same complaint, which is particularly liable to occur in kala-azar patients.

According to Adler and Tchernomoretz (1939), 4 : 4′-diamidino stilbene in toxic doses produces extensive renal damage in hamsters. For this reason particular attention was paid to the condition of the urine during treatment. Some evidence of renal damage—albumin, blood, and even casts in the urine—is a common finding in cases of Sudan kala-azar on admission to hospital; but as a general rule the condition of the urine improved during treatment with 4 : 4′-diamidino stilbene. In case no. 4, however, the patient died with signs and symptoms suggesting severe renal damage. But it may be noted that not only was this patient practically moribund on admission, but there was evidence suggestive of renal involvement from the beginning, and the patient had had in addition a single large dose of neostibosan. The amount of 4 : 4′-diamidino stilbene given was small, and, in view of the much larger doses tolerated by other patients without accident, it is very doubtful whether this death can be attributed to a toxic action of the drug on the kidney.

In cases no. 3, 5, 6 and 7 there occurred during treatment a series of reactions which can best be described as an exacerbation of the signs and symptoms of the disease. Except in case no. 6, this was associated with a very noticeable increase in the splenic enlargement. The significance of it is obscure, but analogy with the Herxheimer reaction is suggestive.

The total dosage required for cure appears to vary within wide limits,

according to the case. In the Sudan, this is true also of antimony. The smallest total dosage of 4 : 4′-diamidino stilbene was given to case no. 7, who was apparently cured after 24 injections of 975 mgm. distributed over five weeks. A case in the later group, who was at first thought to be resistant, required treatment extending over six months and involving more than 70 injections (4·4 gm.) before a satisfactory effect on the disease became evident. Probably also the time-distribution of the dosage is important. Little information is as yet available on the excretion of 4 : 4′-diamidino stilbene, so that observations on this subject are at present empirical. Our results suggest that daily dosage is more effective than dosage on alternate days, and favour an intensive execution of the treatment scheme; but the optimum dosage and scheme of treatment have still to be worked out by trial and error. When these have been determined, it may be possible to reduce the period of hospitalization, which averaged 15 weeks in the six successful cases in the present series.

REMARKS ON THE TREATMENT OF SUDAN KALA-AZAR

In assessing the value of a new drug in the treatment of kala-azar, the ultimate results, including relapse, must be considered, as well as the immediate results. In the case of 4 : 4′-diamidino stilbene time alone can show what the ultimate results will be, but the immediate results are encouraging, and in the opinion of the writers do not compare unfavourably with those obtained previously in this country with trivalent and pentavalent antimony compounds. It is admitted that they compare unfavourably with the results reported from India, where apparently kala-azar can be cured in eight days with certain of the drugs at present in use, and some comment is required on this point. Detailed references to the literature are unnecessary here, since Schmidt and Peter (1938) have compiled an exhaustive summary of the literature from different countries relating to treatment by antimony, which gives an excellent bird's-eye view of the subject, and to which reference should be made.

From such a survey it is apparent that there are real differences in the severity of kala-azar and in its reaction to treatment in different places. Indian kala-azar seems to be much more amenable to treatment with antimony than the other varieties of the disease, but the mechanism of its reaction is obscure. Complete clinical cure is apparently consistent with an extensive invasion of the skin by the parasites, where they may at a later date produce cutaneous lesions without causing a relapse of the visceral disease. In Chinese kala-azar the action of antimony appears to be much slower, while the clinical picture of the disease is graver—agranulocytosis and complications affecting the mouth, throat and lungs are specially frequent and severe. In addition, Chinese patients seem particularly sensitive to antimony, so that toxic reactions are common, the death-rate is high, and prolonged hospitalization is necessary for cure. The tartar emetic treatment, which was at one time used extensively in India,

apparently never met with any degree of success in China. Even with the pentavalent compounds, it has proved difficult to adopt a standard scheme of treatment as in India, and modifications are frequently required to suit individual cases. Mediterranean kala-azar also appears more difficult to influence with antimony than the Indian variety. Here, also, the Indian standard treatment has proved inadequate, and larger doses are generally required. The special difficulty most frequently mentioned in papers from the Mediterranean region is antimony-resistance. Some workers distinguish between primary and secondary or induced resistance, and state that intensive execution of the treatment scheme is essential to avoid secondary resistance.

Little has been published on the treatment of Sudan kala-azar, but it may be stated that this combines the difficulties of both Chinese and Mediterranean varieties. The clinical picture of the uncomplicated disease is a grave one. Intractable diarrhoea, haemorrhages, and complications like cancrum oris and lobar pneumonia are frequent. So also are superimposed infections, of which helminthiasis, malaria, amoebic and bacillary dysentery are the commonest. Experience with antimony has shown that the Indian standard treatment is inadequate in the Sudan disease, where the action of the drug is much slower. A larger total dosage is usually required to effect a cure, and cases are frequently encountered which are completely resistant to any form of antimony treatment. At the same time, Sudanese kala-azar patients are very sensitive to the toxic effects of antimony, and the early attempts to treat the Sudan disease with this drug along the lines advocated by experienced workers in India led only to a series of disasters, which Archibald (1923) recognized as attributable to the drug rather than to the disease. Principally for these reasons, no standard treatment has been evolved for Sudan kala-azar. The best results are obtained where the physician is ready to modify his scheme to suit individual cases, and vary with individual clinical experience. Few workers in the Sudan can claim as good results as those reported by Henderson (1937), who treated 300 cases in four years : the mortality in the first year was 50 per cent., but in the latter part of the series it was reduced to 25 per cent. The average period of hospitalization in successful cases was three months. Even these results compare unfavourably with those reported from other countries, and do not take into account the relapse-rate, which is difficult to assess accurately in a country like the Sudan but which is considerable.

These factors must be considered in assessing the present results. It would appear already that similar divergencies occur in the reaction of the different varieties of kala-azar to treatment with 4 : 4′-diamidino stilbene. Thus, Adams and Yorke (1939) obtained an immediate cure in a case of Indian kala-azar after eight daily injections of 1 mgm. per kilo., while the case of Mediterranean kala-azar treated by Adler and Rachmilewitz (1939) required 27 injections of 1·7 mgm. per kilo. The total dosage in both instances is less than the average total dosage which we have found necessary in the Sudan.

SUMMARY

1. Case-histories are given of eight cases of Sudan kala-azar treated by intravenous injections of 4 : 4′-diamidino stilbene.

2. There were two deaths, and six apparent recoveries which have shown no tendency to relapse during an observation-period of four months. The patients who died (cases no. 1 and 4) were moribund when treatment commenced, and died after they had received respectively only three and five doses of the compound.

3. Reference is made to 20 additional cases treated more recently.

4. With doses varying from 1 to 2·6 mgm. per kilo. body weight toxic reactions have been negligible.

5. The total dosage required to effect an apparent cure varies considerably with different cases.

6. In assessing these results due importance must be given to the difficulties encountered in the treatment of Sudan kala-azar, which is compared in this respect with other varieties of visceral leishmaniasis.

ACKNOWLEDGEMENTS.—The writers are indebted to Professor Warrington Yorke for supplies of 4 : 4′-diamidino stilbene, and for his kind advice and guidance during the whole course of this work; to Dr. E. S. Horgan and Dr. N. L. Corkill, for help and facilities to carry out the work; and to Dr. E. D. Pridie, Director of the Sudan Medical Service, for facilities, for the interest which he has taken in the progress of the work, and for permission to publish these results.

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A NEW PLAIN-WINGED *ANOPHELES* FROM RHODESIA

BY

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In January, 1940, Dr. J. Muspratt, of Salisbury, Southern Rhodesia, sent to the Liverpool School of Tropical Medicine specimens of an anopheline mosquito which he considered might represent a new variety of *A. rupicolus* Lewis. Dr. D. S. Bertram, who examined the material, also concluded that it represented something new, but desired me to investigate the matter and prepare a description. This I have gladly done, and should like to express my indebtedness to Dr. Bertram and Professor Gordon, as well as to the collector, Dr. Muspratt, for the opportunity of placing on record this interesting and most unexpected discovery.

A second consignment was received from Dr. Muspratt in April, 1940, including numerous adults and very perfect mounts of larvae and pupal pelts in gum chloral. Dr. Muspratt states that the adults were all bred out in captivity, so that there is no question as to the larvae from which they come.

The specific name, suggested by the collector, has reference to the habitat of the larvae.

Comparison with the larvae of *A. rupicolus* is rendered simple on account of the very full and illustrated description given by Salem (1938), who redescribed *A. rupicolus* as a new species, *A. aegypti*.

Anopheles (Myzomyia) ruarinus sp. nov.

ADULT. A dark species with unspotted wings, resembling *A. rupicolus* but much larger, the wings measuring 4·5–5 mm. in length in ♀ and 3·8–4·2 mm. in ♂.

♀. *Head.* Interocular space of average width, clothed with a few dull whitish scales and pale bristles forming a very inconspicuous frontal tuft. Erect scales of vertex moderately expanded, nearly all blackish, only a few whitish scales forming an inconspicuous patch in front. Antennae devoid of scales. Palpi uniformly slender, scales dark and appressed, no trace of pale rings at joints; index about 0·35. Mandible with about 30–35 teeth on the expanded end.

Pharynx. Of the *Neomyzomyia* type, with 10 teeth (including a small one on each side), each with a strong denticle on each side at base and rather numerous small denticles scattered over most of the surface, the whole tooth appearing

more jagged than usual. The four ventral papillae well separated. No spicules on pharyngeal ridges.

Thorax. Integument rather shining and mainly pale-brownish, but posterior part of mesonotum often appearing dark, leaving fossae pale. No scales whatever present, unless a few slightly thickened hairs on front margin can be reckoned as scales. No definite markings of any sort. Two or three propleural bristles ; no spiraculars.

Abdomen almost entirely dark-brown, devoid of scales.

Legs uniformly dark-brown except for the pale yellowish coxae ; no trace of pale knee-spots.

Wings entirely dark-scaled, without any trace of pale spots even on costa in any of the numerous specimens examined, thus differing from those of *A. rupicolus*, which, as stated by Lewis, have usually two faint pale areas on the costa. Forks short, stem of upper almost as long as the cell.

♂. Resembles ♀. Palpi entirely dark, with slender club.

Terminalia of the usual *Myzomyia* type, structure much as in *A. rupicolus*, but two hairs present instead of one between the long terminal hair of the harpago and the club ; tip of club more rounded and scarcely curved inwards. Phallosome with five pairs of leaflets, the longest pair serrated on one edge.

PUPA. Four pelts examined differ markedly from the pupal pelt of the type of *A. rupicolus*, as well as from Salem's figure of the pupa of *A. aegypti* in the shape of the paddles and some other details.

Paddle broadest a little beyond the middle, the outer margin then running almost straight to the somewhat pointed tip ; outer margin almost bare on proximal half, with longish fine fringe on distal half ; inner margin entirely bare. Midrib curved, faint but running to tip. Hooked seta placed at the tip, accessory seta far from it.

Spine A : VIII, more than 1/2 length of segment and much branched ; VII, nearly 3/4 length of segment ; VI, over 1/2 ; V, about 1/3 length of segment ; IV, very short. Hair B : IV-VII, with 3–5 branches, 1/2 length of segment or not much more. Hair C : V-VII, simple and somewhat longer than segment ; IV, branched and about 1/2 length of segment.

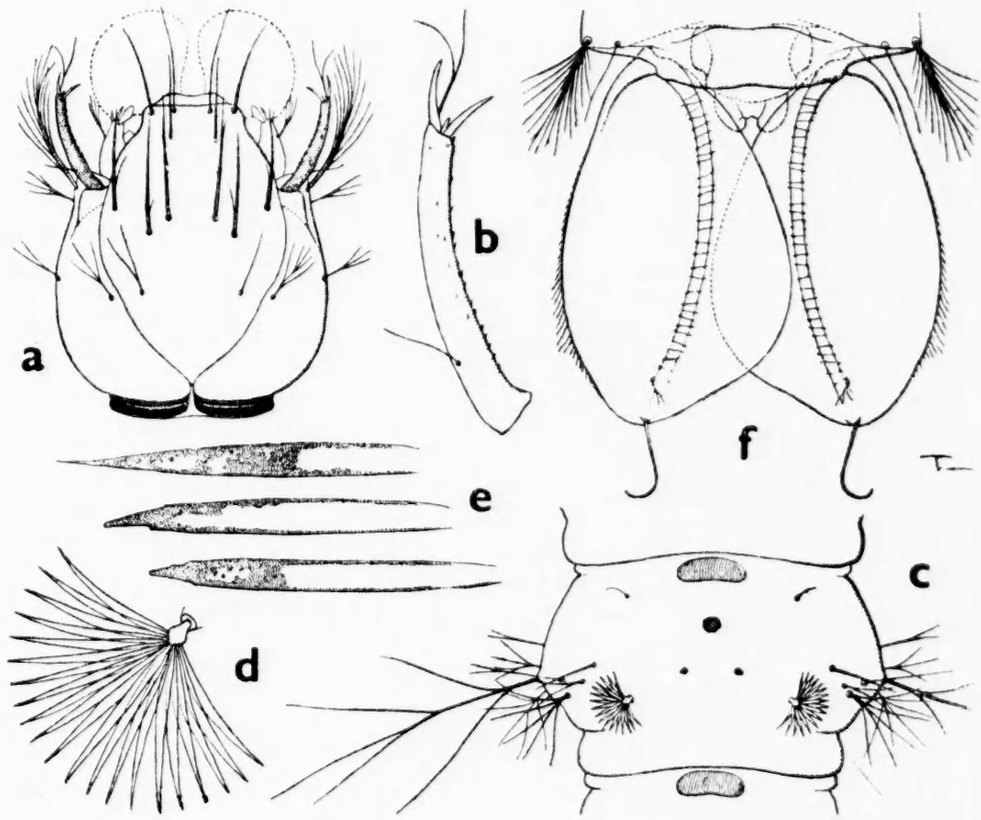
LARVA. Differs notably from most or all other African anopheline larvae in having the frontal hairs simple or almost so ; the larva of *A. rupicolus* has normal plumose frontal hairs, and also differs in many other respects. The description below is based on a comparison of numerous whole larvae and several pelts.

Size large ; length, excluding caudal setae, 11–12·5 mm. (spirit and gum chloral specimens). Colour in life, according to collector, dark greenish-grey.

Head. Very dark, almost black in colour, so that the usual markings are obscured (in contrast with *A. rupicolus*, which has a paler head with obvious markings). Antennae wholly blackish and rather strongly curved—two very unusual features ; inner surface with small, scattered, nearly uniform spicules,

outer surface almost smooth. Clypeal hairs all simple, the posterior pair at least 2/3 as long as the inner and somewhat stouter, relative positions normal. Frontal hairs : inner and middle usually entirely simple, sometimes with a single short branch at base ; outer usually with 1–3 short branches at base but sometimes quite simple, all rather stout. Post-frontal hair simple or bifid, vertical simple or with 2–5 branches.

Thorax. Shoulder hairs well separated, inner and middle each with about 10 branches, inner with the chitinized base very small, scarcely visible. Palmate



Anopheles ruarinus sp. nov.

a.—Head of larva. b.—Larval antenna. c.—Fourth abdominal segment of larva. d.—Float hair. e.—Leaflets of float hairs enlarged ; normal form above, variations below. f.—Pupal paddles.

hair not developed, represented by a small tuft of 3–6 branches which are at most very slightly flattened. No small plates on metathorax. Pleural hairs : pro-pleural with two long simple and one feathered ; meso- and meta-pleural, one long simple and one long feathered.

Abdomen. Palmate hairs : I rudimentary, with about 6 almost hair-like leaflets ; II-VII well developed, but II and VII less so than the others, all with about 18 leaflets which are usually narrowly lanceolate and sharply pointed, without trace of shoulder or serrations, though in one or two specimens a slight

shoulder and a few serrations are present, and in these the leaflets are shorter and less pointed, but not at all resembling those of *A. rupicolus*. Tergal plates small (relatively to the size of the larva they are much smaller than in *rupicolus*), occupying less than 1/3 of the total width of the abdomen. Median accessory plates present on II-VII (very small on II) and small paired posterior accessory plates on I-VII. Pecten with only 3–4 long teeth and 6–8 short ones. Saddle hair long and simple (as is probably normally the case also in *rupicolus*, though in the type-pelt it is double). Gills long, more than twice as long as saddle (in *aegypti* = *rupicolus* according to Salem they are only 3/4 as long as saddle).

EGG. Unknown.

BREEDING-PLACE. ' Larvae were collected from rock pools on the top of a flat rock Kopje which is termed a " ruari " by the natives. The pools had a small amount of muddy sediment at the bottom, but few weeds. Other larvae present were *Aëdes vittatus* and *Culex vansomereni* ' (note by collector).

DISTRIBUTION. All the material described was collected on the Ruia Estate, P.O. M'Sonneddi, Salisbury, Southern Rhodesia, in January and March, 1940.

Types and other specimens presented to the British Museum by Professor R. M. Gordon and Dr. D. S. Bertram, paratypes in the Liverpool School of Tropical Medicine.

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THE EFFECT OF A DIET OF HUMAN BLOOD ON *TRYPANOSOMA BRUCEI* DEVELOPING IN *GLOSSINA TACHINOIDES*

BY

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In his enquiry into the Mwanza epidemic, Duke (1923) considered that *T. brucei* obtained a footing in man owing to (1) a great decrease in wild game, which caused the fly, *G. swynnertoni*, to turn to man for its food, and (2) a simultaneous weakening of the human population owing to famine and ankylostomiasis. It occurred to the present author that, if this is the true explanation of the epidemic, the essential factor may have been the acclimatization by developing forms of *T. brucei* in the fly's gut to human blood repeatedly imbibed by the fly. By chance, some flies, after only one or two meals on game, may have fed on man alone for the remainder of the period necessary for cyclical development of the trypanosome. In this way, it may be argued, the trypanosomes developed a resistance to normal human serum and became able to infect man ; and the failure of the many attempts that have been made to infect man with *T. brucei* may be due to the fact that no attempt has first been made to develop a resistance in the trypanosomes to normal human serum. The experiment which follows was designed to test this hypothesis, but, before it is described, it is advisable to summarize briefly some of the possible flaws in the argument just given.

Firstly, Duke provides no real evidence that the Mwanza epidemic was in fact derived from game and not from an unsuspected human source. Secondly, the rate of destruction of the trypanocidal substance of human blood in the fly's gut is unknown ; complement is known to be rapidly destroyed (Adams, 1931). Thirdly, the peritrophic membrane prevents actual contact between the imbibed blood in the gut and the trypanosomes developing in the peritrophic space, though by analogy with haemoglobin, to which substance Wigglesworth (1929) has shown the membrane to be freely permeable, the trypanocidal substance, which is associated with the globulin fraction of the serum (Culbertson, 1935), might also be expected to pass freely. Fourthly, the resistance of man to infection by *T. brucei* may be entirely unconnected with the trypanocidal power of his serum (Adams, 1933) ; in this connection it is relevant to observe that *T. brucei* is capable of culture in a medium half of which consists of citrated human blood (Brutsaert and Henrard, 1936). The strain used in the present experiment cultured readily in such a medium made with the writer's blood and sown within a few minutes after removal of the blood from the

subject to ensure that none of the trypanocidal substance was lost by standing. This indicated that some of the trypanosomes at any rate can withstand human blood indefinitely at room-temperature. Finally, Duke himself (1935), using tsetse infected with a strain of *T. rhodesiense* which had lost the power of infecting man, found that feeding the fly on human blood for the first three weeks of cyclical development did not enable the flies eventually to transmit the infection to man.

It thus seems unlikely that feeding tsetse on human blood would influence the infectivity for man of the *T. brucei* developing in their gut. But in view of the importance of the question, of the opinion held by perhaps the majority of workers that the trypanosomes of man must in some way and at some time have been derived from *T. brucei*, and of the failure up to the present to demonstrate how this step might have occurred, it appeared worth while making the experiment. Accordingly, pupae of *G. tachinoides* were collected and hatched out in the laboratory, and a strain of *T. brucei* was obtained in guinea-pigs. This strain had been recently isolated from a naturally infected horse by Mr. R. S. Marshall, veterinary pathologist, and sent by him to the author in guinea-pigs ; it was found to have a fairly high virulence for these animals, the blood infection usually progressing steadily, with only incomplete remissions. It was also found to have a high resistance to reduced tryparsamide *in vitro*, requiring concentrations of about 1 : 1,000,000 to kill 100 per cent. of the organisms in 24 hours at 37° C. All trypanosomes disappeared in pure human serum in about six hours.

Batches of the newly hatched flies were given either one or two feeds on guinea-pigs infected with the strain, and then daily feeds on healthy volunteers commenced. It was intended to continue the feeds on volunteers until the cycle was completed, but unfortunately at this stage the authorities decided to close Gadau laboratory, where the work was being conducted, and to send the author home on leave. The original plan had therefore to be abandoned, and it was decided to limit the experiment to a comparison of the parent and fly-transmission strains in regard to their resistance to human serum in guinea-pigs. To avoid risk to the volunteers, whose health could no longer be supervised, their use was discontinued in time to prevent possible infection, and shed citrated human blood was substituted. The flies were kept at 33–35° C. between feeds when the incubator was not in use for other work, in order to hasten cyclical development, and, when cyclical development was judged to be nearing completion, were fed on clean guinea-pigs. These pigs accompanied the author on the voyage to England on leave, and the modified experiment was completed on board an Elder Dempster steamer.

EXPERIMENT

Thirty-two newly hatched *G. tachinoides* were fed for 2 days on a guinea-pig infected with the parent strain, for 6 days on a healthy volunteer, then for 7 days on citrated human blood contained in a beaker covered with fresh vulture-skin, and finally for a further 7 days on a clean guinea-pig (T). Eighteen flies then still survived, of which 10 were

dissected. Three of these 10 flies showed salivary gland infections. Trypanosomes were first seen in the blood of pig T about a week after the last feed. Thereafter the blood was examined every second or third day and trypanosomes were always found, there being no evidence of any natural intermission. After remaining at 1 to 5 per field of the 1/6 objective for 10 days, the trypanosomes became more numerous, and on the 14th day were swarming. On this day 4 c.cm. of serum from the author's blood were injected intraperitoneally into the pig, and another 4 c.cm. into a pig (P) carrying the parent strain passed from pig to pig by inoculation of infected blood. Pig P then showed about 10 trypanosomes per field, trypanosomes having first appeared 4 days previously and steadily increased in numbers each day. The dosage of 4 c.cm. of serum was chosen because that dose had previously been found to cause disappearance of trypansomes for one week in a pig infected with the parent strain, the organisms returning on the 8th day. Guinea-pigs T and P were examined after 24 hours, again after 4 days, and again on the 8th day following the administration of serum. No trypanosomes were seen in either animal on any occasion. No further observations were possible.

A crude *in vitro* test was also carried out. Before injection of serum into the guinea-pigs, a drop of blood from the ear of pig T was added to about 1/2 c.cm. of sterile serum in a tube (T1), and a drop from pig P was put up similarly in another tube (P1). Tube T1 was found to contain 40 trypanosomes per 20 fields and tube P1 17 when one small drop of the fluid was placed on a slide and spread uniformly under a slip, the same-sized cover-slip being used in each case. The tubes were left at room-temperature, which varied between 82° and 85° F., and were re-examined after 19 hours. By this time tube T1, which had started with 40 trypanosomes per field, showed only 6 sluggish trypanosomes and some ghosts. Tube P1, rather surprisingly, showed 24 mainly sluggish trypanosomes. Both tubes remained sterile.

CONCLUSION

Feeding *G. tachinoides* containing developing *T. brucei* on human blood did not produce any increase of resistance to normal human serum in the trypanosomes subsequently transmitted to a guinea-pig by the bites of the fly, as far as was shown by injection of human serum into the infected guinea-pig. A crude *in vitro* test appeared to show that the fly-transmission strain was even less resistant than the parent strain.

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A TRIAL WITH 4:4′-DIAMIDINO STILBENE IN THE TREATMENT OF SLEEPING SICKNESS AT GADAU, NORTHERN NIGERIA

BY

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At the beginning of 1939, with the consent of the Deputy Director, Sleeping Sickness Service, Nigeria, Professor Yorke despatched to the author a supply of 4 : 4′-diamidino stilbene for trial. This drug had not hitherto been used on man in Africa, and it was therefore necessary to proceed cautiously in working out the best dosage. For this reason the total dosage given was far less than the maximum which has since been shown by other workers to be tolerated, though in some cases the rapidity of administration for short periods—1 mgm. rising to 2 mgm. per kilo. body weight on alternate days—was probably nearing the margin of safety. Before describing the actual trial, it will be as well to give a brief account of the type of case encountered.

About 1930, the headquarters of the Sleeping Sickness Service was moved to Gadau from Sherifuri, five miles away, where work on trypanosomiasis had already been going on for several years. In 1934 it was again transferred to Kaduna, but a medical officer has been stationed at Gadau, with occasional short intervals, ever since. In addition to the large number of patients who have come to Gadau hospital during all these years to seek treatment voluntarily, a large part of the surrounding population has been systematically surveyed and treated *en masse* by sleeping sickness teams over a number of years. In this way more intensive and continuous treatment has been available to sleeping sickness patients in this area than in any other part of Nigeria. Possibly as a result of this, the disease, which existed in a severe epidemic form 10 years ago, now exists only in a state of low endemicity, with an incidence of well under 1 per cent. over the area as a whole, sporadic cases cropping up here and there, chiefly amongst fishermen and others whose occupations take them frequently to the neighbourhood of rivers and streams. Though of low incidence, the disease is manifested in those seeking treatment in a peculiar and severe form not commonly seen in other parts of Nigeria. As sleeping sickness differs so widely in its manifestations in different parts of Northern Nigeria, and since in the author's opinion it is of the greatest importance when attempting to estimate the value of a new preparation to have a clear knowledge of both the type of disease to be treated and its reaction to drugs already proved, a brief description of the disease as seen at Gadau in 1939 will now be given. The author hopes to publish shortly a fuller account of the unusual symptomatology met with in this region, together with a study of the factors which have been instrumental in bringing it about.

Amongst 100 unselected cases coming for treatment during 1939, enlarged cervical glands were found in only 71, of which 62 showed trypanosomes in fresh gland-juice, blood-films were positive in only 22, but no less than 94 were found to have pathological spinal fluid. The majority of the cases suffered from nervous lesions, as shown by marked somnolence, mental changes, tremor, or ataxia, and in at least 28 per cent. the disease manifested itself chiefly in the guise of a pituitary-thyroid syndrome, as evidenced by adiposity of pituitary type, myxoedema, and often one or more of the following: impotence in men, amenorrhoea in women, retardation of sex-development in boys, and a tendency to bradycardia, where this was not masked by tachycardia attributable to the direct effect of the infection on the heart. Severe itching was a troublesome complaint amongst most of the advanced cases, particularly in those exhibiting myxoedema. From a study of a few cases who were kept under observation without treatment for two or three weeks, and of others treated with diamidino stilbene in whom the disease was not apparently checked, it is evident that the natural course of the disease, once the nervous system becomes definitely involved, is rapid in this area.

RESULTS OF TREATMENT WITH ANTRYPOL AND TRYPARSAMIDE

Taking the typical Gadau case with serious nervous involvement, a standard course of antrypol and tryparsamide comprising 3 1-gm. doses of antrypol followed by 5 2-gm. doses of tryparsamide (all injected at 5-day intervals) caused considerable clinical improvement and some amelioration of the C.S.F. picture; this improvement commenced during the administration of antrypol and continued as the administration of tryparsamide proceeded. When tryparsamide was given from the start, the initial improvement was even more rapid. The standard course, however, was quite insufficient to prevent relapses, and tryparsamide was usually continued for a long period, in an effort to obtain permanent cure. Taking the average of some two dozen patients, the course of whose disease was carefully studied by serial lumbar puncture, the findings may be summarized as follows. The average patient on admission presented the symptoms already described; lumbar puncture revealed something over 100 cells and a moderate increase of globulin by the saturated ammonium sulphate ring test. After 10 weeks, during which period he had received some 4 gm. of antrypol and some 14 gm. of tryparsamide, the patient had become symptomless, and his C.S.F. revealed only a slight increase of globulin and between 20 and 30 cells. After continuation of tryparsamide for a further five weeks the C.S.F. picture appeared to be stationary, showing a faint cloud of globulin, 27 mgm. of total protein by Sicard and Canteloube's method, and 15 cells, and treatment beyond this point did not alter the picture. No cases thus treated returned with a relapse during periods up to 13 months after discharge, but, as proper following-up was impossible, a final verdict as to cure cannot be

given. It may be said, however, that patients treated with the short standard course only frequently returned in a relapsed condition after a few months.

TREATMENT WITH DIAMIDINO STILBENE

The first batch of the drug was received early in 1939, and further batches, packed in ampoules of 50 or 100 mgm., were sent as suitable cases turned up for treatment. As the patients at Gadau were mostly of the advanced type, it was later arranged that Dr. McLetchie should carry out a trial with the milder cases which he was finding in a survey in the south of Bauchi Province. His results are published in another paper in the present number of this journal.

Dosage Employed

Thirteen cases were treated in all, excluding one case who absconded before a second lumbar puncture could be performed. Five of these cases received two or three injections of the drug intramuscularly into the buttock in doses of 100 mgm. on alternate days. The remainder were treated with a varying number of intravenous injections in doses varying from 50 to 100 mgm. for adults, given either on alternate days or once in three or four days. The lowest total dosage any adult patient received was 170 mgm., and the highest 550 mgm.

Progress of Cases

Trypanosomes were found to disappear from blood and gland-juice after either the second or third injection, i.e., in three or four days. Most cases showed an almost immediate clinical improvement and lessening of symptoms, though the condition of others was hardly influenced; where improvement did occur it was frequently only temporary, and after a week or two the patient's general condition again declined. When administered intravenously the drug caused some epigrastric discomfort in many cases, vomiting on two or three occasions, and collapse in one case after an injection of 2 mgm. per kilo.; the patient in this case became unconscious and pulseless for half a minute, but he recovered very rapidly and was normal again in a few minutes. Many injections of 2 mgm. per kilo. were, however, given without causing any complaint.

The main points have been summarized in the table. Case 84, who started with a normal C.S.F., was apparently quite cured. Case 44 was also clinically cured and felt perfectly fit, but lumbar puncture still revealed 10 cells. There is little doubt that antrypol or tryparsamide would also rapidly have cured these two early cases. Case 51 was also clinically cured, but his cell count remained so far from normal that one must entertain some doubt as to his ultimate fate. Case 45 improved clinically, but his cell count had increased slightly. Of the remaining nine advanced cases, one was no better and his C.S.F. remained very abnormal, while eight were definitely worse after treatment and their C.S.F. picture had deteriorated. A living trypanosome was found in the C.S.F. of case 82

after she had received a total dosage of 13·8 mgm. per kilo. Some of these patients deteriorated so far that they would undoubtedly have died if treatment with antrypol and tryparsamide had not been instituted, which is the reason why the period of observation shown in the table is sometimes so short. Under antrypol and tryparsamide they soon improved again.

TABLE

Showing results of treatment with diamidino stilbene

	Before treatment					After treatment		
Case no.	Clinical condition	C.S.F.		Total drug : mgm. per kilo.	Interval after commencing treatment	Clinical condition	C.S.F.	
		Cells	Globulin				Cells	Globulin
44	M.20. Mild case ; not somnolent	16·2		3·2 intramuscularly	3 mths.	Cured	10	
45	M. 23. Moderately severe; somnolent	30		5·0 ,,	5 wks.	Better, but not cured	74	
50	M. 21. Severe case; somnolent	292	—	6·3 ,,	2 wks.	Worse	1,897	++
51	M. 17. Moderately severe; somnolent	191		6·0 ,,	1 mth.	Cured	83	—
56	M. 23. Severe ; somnolent	442	+	5·0 ,,	3 wks.	Worse	551	++
59	M. 26. Severe ; very somnolent	514	+	7·0 intravenously	1 mth.	Worse; incontinent	642	+++
60	M. 8. Severe ; somnolent	342	++	4·8 ,,	2½ wks.	Moribund	200	
66	M. 19. Moderately severe; slight somnolence	415		7·3 ,,	2½ wks.	No better	248	+
72	F. 27. Moderate ; not somnolent	87	sl+	7·2 ,,	1 mth.	Worse	98	++
80	M. 19. Moderately severe; somnolent	181	+	9·5 ,,	1 mth.	Worse	355	+
82	F. 25. Mild case ; somnolent	44	—	13·8 ,,	7 wks.	Mad ; trypanosome found in C.S.F.	513	++
*83	M. 55. Moderate ; not somnolent	54	—	10·0 ,,	2 wks.	Worse	163	+
*84	M.19. Moderate; somnolent	3·9	—	8·0 ,,	5 wks.	Cured	3·0	—

*In these two cases treatment was not started until three weeks after the first lumbar puncture.

The general results obtained with similar cases treated with antrypol and tryparsamide have already been given for comparison, but, to make the comparison more graphic, an actual case which has been selected as typical is here described.

CASE 81. Male, aged 34. A moderately severe case, somnolent, dull-witted, with a tremor of the tongue. Lumbar puncture on admission revealed 734 cells and globulin +. After receiving 5·3 gm. of antrypol and 10 gm. of tryparsamide over a period of 8 weeks, the patient was symptomless, but his C.S.F. still showed 36 cells and globulin +. After a further 10 gm. of tryparsamide over 3 weeks, examination revealed that the C.S.F. contained 11 cells, a very faint cloud of globulin, and a total protein of 22 mgm. as estimated by Sicard and Canteloube's method. Completed treatment covered 14 weeks, during which a total of 5·3 gm. antrypol and 24 gm. tryparsamide were administered.

CONCLUSION

Of 13 sleeping sickness cases treated at Gadau during 1939 with diamidino stilbene, three mild or moderately severe cases were clinically cured and one improved, though in no case did the C.S.F., when initially pathological, return to normal. Of the remaining nine moderate or severe cases, one was clinically unchanged and eight were definitely worse, while all showed greater disturbance of the C.S.F. after treatment followed by a varying period of observation. The cases seen at Gadau are, in general, of a severe type and require a long course of antrypol and tryparsamide before apparent cure is obtained; even then the C.S.F. is usually just outside normal limits. Diamidino stilbene is therefore subject to a very severe test when tried out on such cases. Nevertheless, after making allowances for this factor and for the relatively very small doses of the drug employed, the author is forced to conclude that the results are not very encouraging and do not justify the hope that the drug would have been as efficacious as antrypol and tryparsamide, even if used in large doses over a long period. It is possible that the present form of the disease at Gadau, resulting from a severe epidemic giving way in the course of years under intensive treatment to an endemic state of low incidence, is peculiarly unsuited to the drug, and that much better results may be expected elsewhere, as other workers' trials in fact indicate (Yorke, 1940).

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DISTRIBUTION OF FILARIASIS IN TANGANYIKA TERRITORY, EAST AFRICA

BY

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This paper describes the distribution of filariasis in Tanganyika Territory and adjacent areas of East Africa. In Table I is shown a summary of microfilarial surveys recorded in the literature. The writer's own material was obtained mostly from patients in the government hospitals and from prisoners in the gaols of the different stations visited.

DISTRIBUTION ACCORDING TO EXAMINATIONS OF BLOOD FOR MICROFILARIAE

1. DAR-ES-SALAAM. All patients entering the large native hospital (Sewa Hadji) had a routine blood-slide taken at midnight. In the first series, all patients admitted during a period of six months were taken, and the results are shown in Table II. In this group, no distinction could be made between local natives and immigrants. The figures for the women and children are given together. A second series of figures was obtained by taking prisoners or consecutive patients who had been admitted for some non-filarial condition, and by subjecting them to closer oral and physical examination. Those not indigenous to the Dar-es-Salaam district were excluded (Table III). The youngest microfilaria-positive child encountered in East Africa was a girl aged six, whose night-blood contained 120 microfilariae in about 10 mm.^{3} of blood ; she showed no clinical signs of filariasis. In the third series, undertaken for another purpose, an examination was made of the hospital and laboratory staff, medical students, etc. Blood-films were taken between 6.45 and 9.10 a.m. Among 103 adult males, there were 12 positive (11·7 per cent), and among 8 females 1 positive. The percentage found in this group differs too much from other estimations to be correct for the whole district. Probably the most accurate estimations of the microfilarial rate† for the local inhabitants of the Dar-es-

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† Microfilarial rate = percentage of those examined found to have microfilariae in the blood. Mf.+ (microfilaria-positive) indicates that the blood was found to contain microfilariae (*bancrofti*).

Salaam district are (*a*) the average of those given in Table III for male patients and prisoners (29·4 per cent.), and (*b*) the figure for females (Sewa Hadji) (18·2 per cent.).

In order to study the relative intensity of the infections, as shown by the number of microfilariae per unit volume of blood, a record was kept of the number of microfilariae per film observed at the Sewa Hadji hospital during a

TABLE I

Summarizing the results of microfilarial surveys recorded for East Africa

District and type of persons examined	Time of examination	No. examined	Percentage carrying *Mf.* *bancrofti*	Percentage carrying *Mf.* *perstans*	
		ZANZIBAR			
Native hospital	8.30–9.30 p.m.	150	38·7	—	Carment (Zanzibar, Ann. Med. Rep., 1915, 32)
University mission hospital... ...	8.30 p.m.	788	31·6	—	Howard (1918)
Villages and Zanzibar town ...	9-10 p.m.	595	33·4	—	Mansfield-Aders (1927)
Pemba...	? night	50	40	—	Dunderdale (1921)
Adults, Weti	9.30 p.m.	156	23·1	—	McCarthy (1930)
Children, Weti	7.30 p.m.	58	15·5		„
		TANGANYIKA TERRITORY			
Bukoba	? day	6,000	—	24–86	Feldmann (1904)
„	„	—	—	51	M.-B. 1910/11, 136
Dar-es-Salaam : soldiers, 1914 ...	night	297	32·3	—	Engeland (1920)
	day	„	2·02	—	„
„ routine blood-films	day	768	6·7 (? *bancrofti*)		Corson (1925)
„ patients, Sewa Hadji hospital	night	100	22	2	Tanganyika Med. Rep., 1925, 121
„ employees, Publ. Health Dept.	7.0 a.m.	100	33	1	
„ school-children	10–11 a.m.	140	5·7	0·7	Corson (1925)
„ prisoners	„	140	5	3·6	„
Kahama	? night	?	20		Tanganyika Med. Rep., 1929, 164
Liwale...	day	?	4	40	Dye (1926)
Liwale-Kilwa area : men	day	357	—	31·4	Irvine (Tanganyika Lab. Rep., 1924, 42)
Mbemkuru River : men	day	179	—	43·0	
Mafia	10 p.m.	169	32·6	0·5	*Ibid.* (1928, 10)
Mwanza : prisoners...	? day	110	11·8	8·2	Engeland & Manteufel 1911)
Rungwe : school-children	midnight	—	32	—	Fischer (1932)
		KENYA			
Lamu, Siyu and Faza	? night	118	35·6	—	Report by M.O., 1911
Lamu, etc., Tana River and along coast	after 8 p.m.	317	35·3	0	Dunderdale (1921)
Nairobi : Askaris, etc.	? day	50	0	0	Greig & Wiggins
„ prisoners from many parts	night	200	2·5	2·5	Lowsley & Ross (1910)
„ „ „ „ ...	9-11 p.m.	423	0·2	1·6	Nairobi Lab. Rep., I

period of four months (January to April). The amount of blood taken for these films was approximately 20 mm.^{3} The results of 194 films were as follows :

1–2	microfilariae per film :	25	cases, i.e.,	12·9	per cent.	of	total.
3–4	,, ,,	17	,, ,,	8·7	,,		,,
5–10	,, ,,	55	,, ,,	28·8	,,		,,
11–21	,, ,,	36	,, ,,	18·5	,,		,,
22–46	,, ,,	28	,, ,,	14·4	,,		,,
47–100	,, ,,	22	,, ,,	11·3	,,		,,
101–210	,, ,,	11	,, ,,	5·7	,,		,,

The most intense infestations encountered (during a different period) were two men with 476 and 464 microfilariae per 20 mm.^{3} respectively. On

TABLE II

Showing the proportions of persons carrying *Mf. bancrofti* and *Mf. perstans* among the total admissions to the Sewa Hadji hospital, Dar-es-Salaam, Nov., 1937, to May, 1938

Series	Total no.	Carrying *Mf. bancrofti*		Carrying *Mf. perstans**	
		No.	Percentage	No.	Percentage
African men ...	1,543	364	23·6	3	0·2
,, women and children	234 (women ?166) (children ?68)	19	8·1 (women ?10·8) (children ?1·5)	1	0·4
Arab men† ...	24	1	4·2 ?	0	0 ?
Indian men	80	7	8·8	0	0
,, women ...	22	0	0 ?	0	0 ?

*The figures for *Mf. perstans* may be too low.
†This may include some Africans of a higher social type, e.g., government clerks.

various occasions studies were made regarding the nocturnal periodicity of *Mf. bancrofti* ; this was always present, unless the patient was moribund or was abnormal in his habits regarding sleep.

2. OTHER PARTS OF THE TERRITORY. In other parts of the country, blood-films were taken from most of the adult natives in the government hospital or local gaol. Blood-films were made between 10 p.m. and midnight, the making, staining and examination of the films being done by the writer personally. No microfilariae other than *bancrofti* or *perstans* were encountered. A man was considered a local inhabitant only if he had lived seven years or more in the locality. Cases admitted to hospital for some filarial condition (hernia here considered as non-filarial) have been excluded. Children under 15 years were

also excluded. In Table IV, the natives of any one place, e.g., Mafia, are shown in various columns: the first, 'examined locally,' gives the number examined at Mafia; the next, 'examined elsewhere,' refers to natives of Mafia who had recently migrated to other districts, and who were encountered in the microfilarial surveys there.

DISTRIBUTION ACCORDING TO HOSPITAL RECORDS

The evidence for the distribution of filariasis as shown by microfilarial surveys was supplemented by that obtained from hospital registers. The figures for filarial conditions have been restricted to patients admitted for hydrocele and elephantiasis (conditions of which the diagnosis is unmistakable); patients with these conditions, admitted for some other reason, are presumably not

TABLE III

Showing the incidence of infestation by *Mf. bancrofti* among indigenous Africans of the Dar-es-Salaam district, Arabs and Indians; examined mostly at the Sewa Hadji hospital after admission for non-filarial conditions

Series	No. examined	Carrying *Mf. bancrofti*		Remarks
		No.	Percentage	
African males:				
(*a*) patients...	56	13	23·2	6, elephantiasis (6%)—2 Mf.+
(*b*) prisoners (6.30-7.30 a.m.)	46	17	37·0	18, hydrocele (17·6%)—4 Mf.+ 1, varicocele (1%)—Mf.+
African females:				
(*a*) Sewa Hadji	77	14	18·2	1, elephantiasis, leg—Mf. +
(*b*) maternity clinic ...	24	8	33·3	May include immigrants
African children, < 15 years ...	51	4	7·8	Age-analysis (see Table IV)
Arabs, etc.	15	3	20?	
Indians, males, in E. Africa:				
> 6 years	37	3	7·4	7, hydrocele – 2 Mf. +
< 6 years	17	1		1, varicocele—Mf. –
Indians, females	17	0	0?	

included; cases of inguinal hernia—another easily diagnosed condition, the relation of which to filariasis is less clear (see below)—have been shown in a separate column. Obviously the figures are affected by many local circumstances, so that only a rough indication of the distribution of filarial conditions can be expected from them. Comparison of these figures with those obtained by microfilarial survey (Table IV) shows that the correlation coefficient between the two sets of data, microfilarial rates and percentages of hospital patients (hydrocele plus elephantiasis; 18 pairs of observations, including some from the literature), is 0·693, and the probability of this being due to chance is 0·001 (Fisher, 1938).

TABLE IV

Showing the distribution of filariasis in East Africa, according to (*a*) microfilarial rates, (*b*) hospital statistics ; and its relation to climatic conditions

District	Men examined		Percentage carrying *Mf. bancrofti*	Women examined	Percentage of total adults carrying *Mf.*		Hospital statistics for in-patients			Altitude in feet	Mean annual rainfall in inches	Mean temperature, °F.	
	Locally	Elsewhere			*bancrofti*	*perstans*	Hydrocele per cent.	Elephantiasis per cent.	Inguinal hernia per cent.			Max.	Min.
Dar-es-Salaam ...	102	—	29·4	77	24·6	0·6	6·73	1·63	6·47	30	42·9	89·7	68·8
Mafia	126	1	36·2	2	35·9	0	3·78	2·83	2·59	64	40·2	—	—
Mombasa ...	0	52*	21·1	12	25·0	3·1	2·20	0·31	3·73	52	53·4	89·3	71·5
Morogoro ...	18	10	46·4?	0	46·4?	3·6?	—	—	—	1,628	37·1	—	—
Iringa	62	4	4·5	11	3·9	0	1·35	0	1·35	5,578	26·3	76·4	56·6
Dodoma	22	2	4·1?	10	2·9?	0	0·27	0·33	0·13	3,675	19·8	83·5	62·4
Arusha	33	6	0	14	0	0	0·47†	0·24†	0·95†	4,416	47·2	74·2	55·5
Moshi	19	7	0	8	0	0	1·06	0·24	1·01	2,649	43·1	79·7	62·1
Singida, Mkalama, Kondoa ...	0	36	0	3	0	0	—	—	—	4,700	29	82	59
Nairobi	86	5	0	0	0	0	0·21	0·02	0·31	5,900	40·2	74·0	52·7
Tabora	23	14	10·8	17	7·4	0	4·03	0·51	2·59	4,035	32·5	83·0	62·5
Kahama	44	5	22·4	7	19·0	0	8·05	3·06	2·48	4,000	35·5	85·3	62·4
Shinyanga ...	186	—	—	—	21·6	0	—	—	—	4,000	28	87	61
Mwanza	57	9	24·2	17	22·9	2·4	16·17	3·75	7·28	3,723	40·9	81·7	62·1
Kisumu	0	32	0	1	0	3·3?	0·09	0·13	0·56	3,734	45·1	81·4	64·2
Kakamega ...	—	—	—	—	—	—	1·33	0·33	4·76	5,100	74·0	80·6	50
Kampala... ...	52	6	0	0	0	46·7	0·24	0·31	5·02	3,905	48·9	79·3	63·1
Bukoba	67	5	0	10	0	41·5	0·45	0·73	4·57	3,740	76·2	80·3	—
Kibondo and Biharamulo ...	0	7	0?	0	0	43 ?	—	—	—	—	—	—	—
Kigoma	61	16	9·1	5	8·7	6·7	1·64	0·49	0·98	2,562	35·5	83·7	66·8
Ruanda-Urundi ...	0	36	0	0	0	19·4	—	—	—	—	—	—	—
Tukuyu	20	4	8·3	18	16·7?	0	3·11	1·31	2·46	5,300	101·4	—	—
Zanzibar	—	—	—	—	32·5	0?	6·70	1·40	7·37	100	62·5	80·3	73·7
Pemba	—	—	—	—	27	0?	10·60	0·57	5·56	100	77·4	86·2	72·8
Lamu, etc. ...	—	—	—	—	44	—	—	—	—	100	37	86·3	77·6

*This includes natives from many parts of the coast : Mombasa, Tanga, Kilwa, Lindi and Utete.
†Indigenous patients only.

Some of the places mentioned deserve annotation :

Mafia Island. Webster and Greening reported that 2·71 per cent. of the population showed elephantiasis ; probably this included only the grosser manifestations (Tanganyika Annual Medical Report, 1925, 121).

Morogoro. The 18 ' local ' cases of Table IV were blood-films kindly taken by Dr. A. Mackenzie from ascertained local natives ; but these seem to have included an unduly high proportion of microfilarial carriers ; probably the true rate is 10–15 per cent. or less. Dr. Mackenzie writes that he has seen very little filariasis in this district which he can be certain is of local origin.

Shinyanga. These figures were kindly supplied by Dr. N. H. Maynard from the Kola Ndota hospital. The prevalence of elephantiasis is noted by MacNaughton (1922).

Mwanza. At the time of the writer's visit, admissions for hydrocele and elephantiasis of the scrotum accounted for 41 per cent. of the local male adult patients.

Tukuyu. The microfilarial rate observed is exaggerated by the inclusion of seven women who were relatives of patients admitted for filariasis or hydrocele ; probably the true rate is about 10 per cent.

Consideration of these figures and those obtained from the literature shows that this part of Africa can be divided into various areas as regards the distribution of *W. bancrofti.*

1. The coastal area, e.g., Dar-es-Salaam (25 per cent.) and Mafia (36 per cent.), including Zanzibar, Lamu, etc., in which the incidence is high ; further inland, e.g., Morogoro, the incidence probably falls somewhat.

2. The region south of Lake Victoria, where the incidence is high at Mwanza (23 per cent.) and diminishes gradually as the distance from the lake increases, through Shinyanga (22 per cent.) and Kahama (19 per cent.) to Tabora (7 per cent.) and Dodoma (? 3 per cent.). Kigoma (9 per cent.) may be considered as belonging either to this area or to the next one.

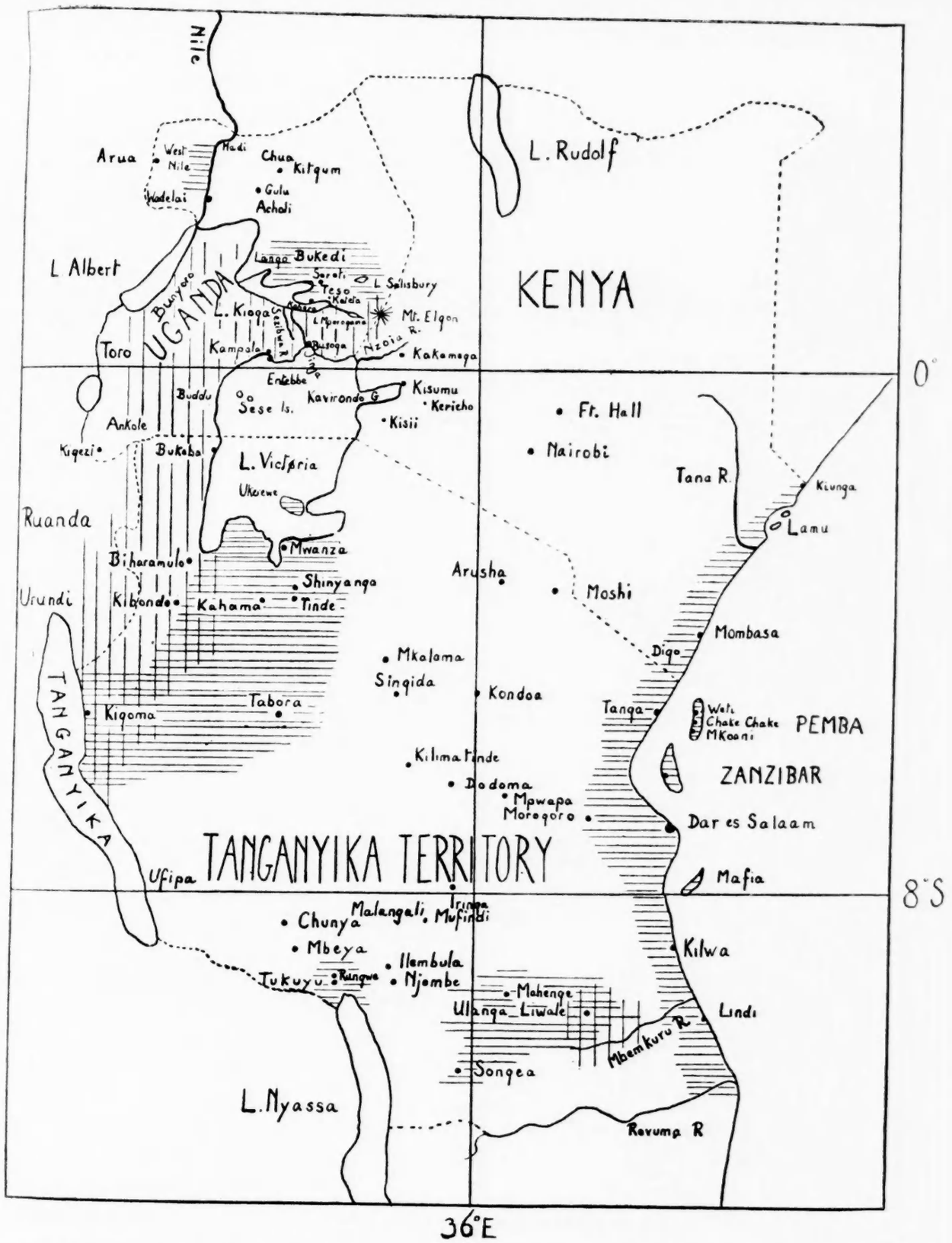
3. Tukuyu (? 10 per cent.), being so separated geographically from the surrounding country, is best considered as a separate area of moderately high incidence.

4. Judging by the literature, Mahenge and Liwale seem to lie in another area of high incidence, the fringes of which may possibly extend to Iringa (4 per cent.) and Songea (Medizinal-Berichte, 1907/8, 100 ; 1908/9, 30 and 78 ; 1909/10, 149 ; 1910/11, 132 ; Tanganyika Territory Annual Medical Reports, 1926, 114 ; 1929, 202).

5. Apparently *W. bancrofti* also occurs in the Teso, Lango, West Nile, Tororo and Ankole districts of Uganda (Uganda Annual Medical Reports, 1937, 11 ; personal communications from Dr. C. J. Hackett, Dr. J. A. C. Spicer and Dr. W. A. Wilson).

6. Finally there is an area apparently free from *W. bancrofti* which commences with the Mkalama-Singida-Kondoa region, extends northwards and westwards through Arusha and Moshi to comprise most of Kenya (Nairobi and Kisumu) and the southern part of Uganda, and reaches round to Bukoba.

A. perstans occurs principally in two areas : (1) The southern portion of Uganda (Christy, 1903 ; Low, 1903), extending to the Kenya border in the east



Map showing the distribution of filariasis in East Africa. The horizontal shading indicates the areas where *W. bancrofti* is prevalent (microfilarial rate > 5 per cent.) and the vertical shading indicates the similar areas for *A. perstans*.

and as far as Kigoma (7 per cent.) to the south-west. (2) The district around Liwale and the Mbemkuru River.

A few apparently indigenous cases appear to occur around Dar-es-Salaam and also at Mwanza and Kisumu, but the remaining portions of Tanganyika Territory and Kenya appear to be free. Apparently the climatic conditions favourable for *bancrofti* are unfavourable for *perstans*, and conversely.

RELATION OF DISTRIBUTION TO CLIMATE

Presumably the distribution of filariasis is determined largely by climatic conditions which control the insect vector. The altitude, rainfall and mean maximum and minimum temperatures of the various places discussed are shown in Table IV, the figures being kindly supplied by the Meteorological Office. When the distribution of filariasis (as shown by microfilarial rates and hospital statistics) is compared with the figures for altitude and for rainfall, no connection can be found ; but with the figures for temperature a definite correlation appears to occur. By statistical analysis it was found that the correlation coefficient between microfilarial rate and mean maximum temperatures (19 places) is 0·524, and that between the rate and the mean minimum temperatures is 0·794 ; the probability of these being due to a chance distribution is less than 0·02 and 0·001 respectively (Fisher, 1938). The similar correlation coefficients for the percentage of filarial patients admitted to hospital (17 places) compared with mean maximum and minimum temperatures are 0·395 and 0·361 (probability = 0·15) ; thus the correlation for the hospital data is less significant, although still suggestive. The most remarkable contrast in the distribution is that between Mwanza (23 per cent.) on the one hand, and Kampala and Bukoba (0) on the other, although these places are in close communication and experience climates which are not widely different. The mean maximum temperatures of the three places are indistinguishable ; clearly other influences are also involved.

CLINICAL LESIONS CAUSED BY FILARIASIS

The occurrence of filarial lesions in natives of East Africa is illustrated by the series shown in Table V ; and the relation of the microfilarial, hydrocele and elephantiasis rates respectively to age is shown in Table VI, which is based upon the figures for children and male adult patients at Dar-es-Salaam (Table III) and the figures obtained on Mafia Island (Tables IV and V). The ages of these patients have been guessed.

These filarial conditions, as they occur in East Africa, may be considered in turn :

1. *Lymphangitis, etc.* The attacks usually agree with the description given by O'Connor and Hulse (1935). Often the patients admit having had similar attacks previously, and sometimes they recur regularly. In a few cases

'focal spots,' as described by O'Connor, could be demonstrated. Elephantiasis is generally present in the regions attacked, especially when this happens to be the scrotum. The figures given in Table V relate to cases which were checked personally. The great majority of persons attacked never come to hospital. The attacks mostly subside quickly under ordinary palliative treatment, but there is no method of preventing their recurrence. No certain case of filarial lymphangitis was observed among Indians or Arabs.

TABLE V

Showing the distribution of filarial lesions (including hernia) among three series of African male adults :

A : Carriers of *Mf. bancrofti* admitted to hospital, Dar-es-Salaam, for non-filarial conditions.
B : Patients admitted to hospital, Dar-es-Salaam, for filarial conditions.
C : Unselected indigenous males on Mafia Island.

	Series A		Series B		Series C	
Lesions absent or indefinite	230	84·9%			132	51·6%
Thickening of spermatic cord or varicocele	33	12·2%	1	1	3	
Femoral lymph-nodes > 2·5 cm. ...	110	40·6%			60	23·4%
Inguinal " " > 2·5 cm. ...	35	12·9%				
Lymphangitis	1		19	12·5% (9 Mf.+)	0	
Hydrocele alone	27	10%	51	33·5% (6 Mf.+)	64	25·0%
Hydrocele plus hernia	2	0·7%	24	15·1% (9 Mf.+)	8	3·1%
Inguinal hernia alone	6	2·2%	37	24·3% (14 Mf.+)	9	3·5%
Elephantiasis	5	1·8%	20	13·1% (6 Mf.+)	40	15·6%
Total	271		152	36 Mf.+	253	

Of the 65 cases of elephantiasis, the scrotum was involved in 53 (including 6 with lymph scrotum), the leg in 23, the arm in 5 ; some men had multiple lesions.

Of the 20 cases with lymphangitis, the scrotum was involved in 10, the leg in 3, the arm in 1, femoral lymphadenitis in 6.

Pyomyositis, i.e., inflammation of the muscles of the limbs, usually proceeding to extensive abscesses between the muscle layers. These abscesses are fairly common in Tanganyika Territory, but there is no evidence that they are due to filariasis.

2. *Chronic adenitis.* In Table V it is shown that, out of 271 carriers of microfilariae, the femoral lymph-nodes were enlarged (> 2·5 cm.) in 110 cases, and the inguinal in 35 cases ; but the majority of these cases may have been due to some other reason. Pronounced enlargement, so that the glands formed great bulging masses of a doughy consistency, did occur occasionally at Dar-es-Salaam, but was comparatively rare ; it seemed to be fairly frequent at Mafia

and Tukuyu. Definite enlargement of the epitrochlear lymph-node was rare.

3. *Thickening of the spermatic cords* is probably filarial in most cases, and occurs commonly in filarial carriers (Table V); but, since its diagnosis is influenced so much by subjective criteria, it has not been listed among the definite clinical indications of filarial disease.

4. *Hydrocele.* This is much the commonest lesion produced by filariasis, and figures for its frequency have been given in Table V, which include all cases in which a definite hydrocele was present, even if small; cases where any degree of elephantiasis of the skin of the scrotum was present have been

TABLE VI

Showing the distribution of *Mf. bancrofti* and filarial lesions according to age among children (< 15 years) and adults examined at Dar-es-Salaam and Mafia

Age in years	Children and male adults		Female adults		Male adults		
	No. examined	Mf.+ per cent.	No. examined	Mf.+ per cent.	No. examined	Cases with hydrocele per cent.	Cases with elephantiasis per cent.
0–3	9	0					
4–6	14	7·2					
7–10	16	6·2					
11–14	16	16			36	0	0
15–25	71	32·4	27	3·7	66	6·1	1·5
26–35	62	41·9	23	26	110	23·6	14·5
36–45	29	24·1	14	21	93	32·2	16·1
>45	23	17·4	13	31	50	40·0	22

classified as elephantiasis and not as hydrocele. As shown in Table VI, its incidence increases steadily with age; after 25 years, 23 per cent. of the men in Dar-es-Salaam and Mafia are affected, and after 45, 40 per cent. are affected. The earliest cases were seen among school-boys 12–14 years old, but at this age it is still uncommon. Occasionally it is accompanied by attacks of inflammation, but generally it seems to develop without pain. The fluids from 82 patients were examined, and microfilariae were present in 10 cases; usually they were alive and motile, but in one case they were all dead, and in many of the other cases in which they were present a few were dead and sometimes partly calcified.

5. *Elephantiasis.* The frequency of this condition is shown in the previous tables. Elephantiasis of the breast and of the vulva respectively was seen in two women at Mwanza.

6. *Lymph scrotum*, i.e., lymph exuding from the surface of the scrotum, was encountered a number of times (Table V). Among six cases seen, the apparent ages were 30, 35, 37, 40, 45, 50. The scrotum was usually somewhat enlarged by elephantiasis. In one case examined, the fluid clotted rapidly unless prevented by citrate ; it contained 3,000 cells per mm.^{3}, of which many were erythrocytes ; of the white cells, 92 per cent. were lymphocytes, 4·5 per cent. monocytes, and 3·5 per cent. of doubtful origin ; no microfilariae were present. The flow of lymph subsided spontaneously in all cases, after a shorter or longer period.

7. *Chyluria.* Two cases were seen ; they will be described elsewhere (Hawking, 1940).

8. *Inguinal hernia.* Although often this is clearly not due to filariasis, in many cases there is an association (probably indirect). The weight of a scrotum enlarged by hydrocele or elephantiasis presumably stretches the structures around the inguinal ring. Statistical evidence of this association is afforded by a comparison between the microfilarial rate and the percentage of patients admitted for inguinal hernia at the different hospitals (Table IV). The correlation coefficient between these two values is 0·577 (16 pairs of observations), the probability that this is due to chance being less than 1 : 50. The frequency with which inguinal hernia occurs in areas where filariasis is endemic has been stressed by Howard (1918) and Knott (1939).

ENTOMOLOGY

During the rainy season, *Culex fatigans* is common in Dar-es-Salaam, being one of the main types of mosquito encountered. In January, 1938, just after the shorter rainy season, 362 specimens of *Culex fatigans* were collected from various parts of the town, and dissected for filarial larvae ; 81 (22·3 per cent.) were positive. Forty-nine mosquitoes (13·5 per cent.) contained early forms, long, thin and active, usually occurring in considerable numbers ; 48 mosquitoes (13·2 per cent.) contained ' thoracic ' forms, thick, stumpy and motionless, usually only one or two per insect ; and 2 mosquitoes (0·6 per cent.) contained mature larvae. The infection-rate seemed to be heaviest in the mosquitoes collected in Kibambawe Street. Seventy-five *C. fatigans* were collected from this neighbourhood and placed in a cage ; at the end of 11 days, 16 were still alive, of which 4 contained mature larvae.

In Zanzibar, Mansfield-Aders (1927) found that 20·3 per cent. of 1,300 wild *C. fatigans* were infected in the proboscis if kept until the 12th day before dissecting. Many *A. gambiae* and *A. funestus* were also infected. In 1929, 1,265 *Culex* from the houses were dissected ; 9 were infected in the proboscis. Twenty-six *C. fatigans* were fed on a patient and dissected 11–19 days later : 7 were infected in the proboscis, 10 in the thorax only, and 9 were negative. In Pemba, McCarthy (1930) found that, of 96 *Culex* dissected, 8 contained filarial embryos ; of 72 *Aëdes*, 3 contained embryos ; and of 58 *Anopheles*, 1 contained

embryos. In Dar-es-Salaam, during the routine examination of anopheline mosquitoes for malaria during 1934-6, Mackay (1938) observed filarial larvae in 0·94 per cent. of *A. gambiae* and in 1·21 per cent. of *A. funestus*.

SUMMARY

1. A description is given of filariasis in Tanganyika Territory and adjacent areas.

2. *W. bancrofti* is endemic (*a*) in the coastal region, the microfilarial rate being about 30 per cent.; (*b*) south of Lake Victoria (microfilarial rate at Mwanza being 23 per cent.), extending to Kigoma and Tabora (rate about 8 per cent.); (*c*) at the head of Lake Nyassa (Tukuyu); (*d*) probably also around Mahenge and Liwale in Tanganyika Territory and the Teso region, etc., in Uganda.

The region commencing around Mkalama, Arusha and Moshi, comprising most of Kenya and the southern part of Uganda and extending to Bukoba, appears to be free from it. This distribution seems independent of the altitude and rainfall, but it can be correlated to some extent with the temperature of the various places.

3. *A. perstans* is endemic north and west of Lake Victoria (microfilarial rate 40–48 per cent.) and around Liwale (rate 30–40 per cent.). Occasional infestations also occur along the coastal region.

4. The main conditions due to filariasis are lymphangitis, hydrocele, elephantiasis of scrotum and limbs (and inguinal hernia); lymph scrotum and chyluria also occur. Among unselected male adults in an area of high incidence (Mafia Island), 24 per cent. had hydrocele alone, 3 per cent. hydrocele plus hernia, 3·5 per cent. hernia alone and 16 per cent. elephantiasis; only 52 per cent. were free from symptoms. The suffering, disablement and economic loss caused by filariasis in certain parts of East Africa are probably very considerable.

5. Transmission occurs through *C. fatigans* and probably also through *A. gambiae* and *A. funestus*.

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THE TRANSFERENCE OF *MICROFILARIA BANCROFTI* INTO NATURAL AND UNNATURAL HOSTS

BY

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This paper describes experiments in which microfilariae (*bancrofti*) were injected into men or animals, in order to study the various hypotheses which attempt to explain the periodicity of these larvae. These hypotheses fall into two main groups, which suppose respectively (*a*) that the periodicity depends upon the adult worms (Lane, 1937); (*b*) that the periodicity is independent of the adult worms, the microfilariae being retained in the deeper parts of the body during the day and reappearing in the peripheral circulation at night.

It was hoped that decisive evidence might be obtained from a study of the behaviour of microfilariae injected into new hosts, and thus isolated from the parent worms, whether they (*a*) were removed from the blood within 24 hours, or (*b*) survived several days and showed nocturnal periodicity.

A.—INJECTION INTO HUMAN SUBJECTS

Blood containing microfilariae was removed from a suitable donor, citrated, and reinjected into a second person whose blood had previously been shown to be free from microfilariae. The search for microfilariae in the blood was greatly assisted by the valuable technique described by Knott (1935): 5 ml. of blood is removed from a vein and mixed with a little citrate to prevent clotting; 1 ml. of blood is placed in each of a series of tubes and 10–15 ml. of 2 per cent. formalin is added, which lakes the blood and fixes the microfilariae. The deposit obtained by centrifuging or standing is spread on a slide, dried and stained.

CASE 1. Emaciated old woman. One enlarged lymph-node, about 1 cm. across, in left femoral region, but none elsewhere. Haemoglobin (Tallqvist) 35 per cent.; R.B.C. 1·8 million, W.B.C. 1,800; poly. 74 per cent.; lymph. 18 per cent.; mono. 7 per cent.; eosin 1 per cent. Blood was taken from a vein at 10.30 p.m., but no microfilariae were found in 6·5 ml. Dirofilarial antigen, 0·25 ml., 0·1 per cent. in saline (Fairley, 1931) was injected intradermally; no wheal was produced. On April 1st, 1938, about 12.15 a.m., 240 ml. of citrated blood containing *Mf. bancrofti* were injected intravenously into the left arm; the transfusion occupied about three-quarters of an hour. The blood given contained 4·2 million microfilariae; there were thus enough microfilariae to give 840 per ml. of her blood, if evenly distributed. A few minutes after the end of the transfusion,

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five slides each of 40 mm.^{3} of blood were made from a finger of the right hand ; these contained 10, 37, 8, 11 and 3 microfilariae respectively, the average being equivalent to 350 microfilariae per ml. Subsequent examinations were made by obtaining 3–6 ml. venous blood, and adding 1 ml. citrate to prevent clotting ; part of the blood was spread as films, each of 120 mm.^{3}, and the rest was divided into a number of centrifuge tubes, and laked with formalin, as described above. The results of these examinations are shown in Table I. On the first day, at 11.30 a.m., four films each of 40 mm.^{3} were made from the finger ; these contained 5, 4, 1 and 2 microfilariae respectively, i.e., 75 per ml. On the evening of the third day, a routine film, taken in mistake by the African attendant, showed 4 microfilariae. After the transfusion, the patient experienced no reaction. She spent

TABLE I

Showing the number of microfilariae found in the venous blood of the recipient, case 1, following the intravenous injection of 4·2 million microfilariae

Time of taking blood	Volume of blood taken : ml.	Microfilariae found		Mf. per ml. of blood (corrected for dilution by citrate)	
		Examined as films(120 mm.^{3})	Examined as deposits after concentration	Slides	Deposits
Start, midnight ...	—	—	—	350 (finger)	840 (calculated)
1st day (Apr. 1st), 11.30 a.m.	6	6, 4, 10, 1, 7	58, 41, 52, 49, 37, 50	54 (finger)	59
10.30 p.m.	6	9, 9, 5, 5, 9	47, 86, 76, 65, 62, 81	82	77
2nd day, 11.45 a.m.	3	2, 5	65, 29, 65	39	56
10.30 p.m.	5	2, 8, 7	61, 65, 77, 64 (1 spoilt)	68	73
4th day, 10.30 p.m.	6	6, 7, 5, 2, 5	38, 33, 37, 39, 30, 19	49	36
5th day, 11.30 p.m.	5·3	3, 2, 0, 4	29, 27, 35, 27, 39	22	34
9th day, 11.30 a.m.	3·5	—	3, 4, 2, 5	—	4
12th day, 10.45 p.m.	5	—	0, 0, 0, 0, 0	—	0

much of the day lying on her bed. On April 4th–6th she complained of pain in the stomach and diarrhoea, and she stayed in bed almost continuously ; on April 11th she improved, and the diarrhoea ceased. On April 16th she was discharged.

CASE 2. Thin old man. Double hydrocele, about 18 × 12 cm. Inguinal and femoral lymph-nodes measured about 1·2 cm. Small right inguinal hernia recurring after operation four years previously. Haemoglobin 40 per cent. ; poly. 75 per cent. ; lymph. 14 per cent. ; mono. 7·5 per cent. ; eosin 3·5 per cent. (April 22nd, 1938). Venous blood, 5 ml., was taken at 10.15 p.m. ; no microfilariae were discovered. Skin-test with 0·25 ml. of Fairley's Dirofilarial reaction gave an ambiguous result. This patient was chosen as having probably undergone infection in the past (indicated by hydrocele) but now showing no microfilariae in the blood. On April 22nd, at 1.0 a.m., 210 ml. of citrated blood were injected intravenously, containing 0·79 million microfilariae ; that would correspond to 157 microfilariae per ml. of the recipient's blood. Five ml. of venous blood were removed 11 and 22 hours later, but no microfilariae could be discovered. The patient experienced a pronounced rigor, commencing 11 hours after the transfusion and lasting about 18 hours.

CASE 3. Man, ? 30 years old. Immigrant from Urundi (where *W. bancrofti* seems rare or absent). Weight 64 kilo. Admitted to hospital for anaemia and ankylostomiasis ; no clinical evidence of filariasis. A skin-test with 0·1 ml. of Dirofilarial antigen intradermally was ambiguous. Five ml. venous blood taken at night contained no microfilariae. At 1.0 a.m. on March 2nd, 1939, 300 ml. of citrated blood were transfused intravenously, containing 0·63 million microfilariae ; that would be equivalent to 126 microfilariae per ml. of his blood. At the conclusion of the transfusion, which lasted three-quarters of an hour, three slides, each of 40 mm.^{3}, were made from a finger of the other arm ; these contained 3 microfilariae (corresponding to 25 microfilariae per ml.). Five ml. of venous blood were taken 11 and 21 hours later ; no microfilariae could be found. Eleven hours after the injection, the patient complained of weakness and headache ; temperature 102·4° F.

TABLE II

Showing the number of microfilariae found in the venous blood of the recipient, case 4, following the intravenous injection of 1·74 million microfilariae

Time of taking blood	Volume of blood : ml.	Microfilariae found in the deposits	Mf. per ml. of blood (corrected for dilution by citrate)
Start, 1.0 a.m. ...			350 (calculated)
5–50 minutes later			69-90 (finger)
1st day 8.30 a.m. ...	5	24, 25, 25, 31, 27	26
(Feb. 9th), 3.0 p.m. ...	5	10, 17, 8, 14, 16	13
10.30 p.m. ...	5	24, 30, 35, 55, 49	39
2nd day, noon	5	31, 52, 45, 49, 31	42
10.15 p.m. ...	4	21, 25, 22, 30, 34	33
4th day, 10.0 p.m. ...	5	38, 51, 40, 60 (1 lost)	47
5th day, noon	5	11, 19, 15, 25, 24	19
8th day, 11.30 a.m. ...	5	18, 31, 14, 14, 12	18
11.0 p.m. ...	5	52, 33, 27, 53, 42	41

CASE 4. Man, about 45 years old, admitted for ankylostomiasis. Weight 58 kilo. Haemoglobin 35 per cent. No clinical signs of filariasis. Five ml. of venous blood, taken at 10.15 p.m., disclosed no microfilariae when examined by Knott's technique. Skin-test with 0·1 ml. of dirofilarial antigen was negative. On February 9th, at 1.0 a.m., 370 ml. of citrated blood were transfused intravenously ; this contained 1·74 million microfilariae, which would provide 350 microfilariae per ml. in the blood of the recipient. Five minutes after the end of the transfusion (which occupied half an hour) four slides each of 40 mm.^{3} were made from a finger of the other hand ; these contained 3, 0, 3, 5 microfilariae respectively, i.e., 69 per ml. Fifty minutes later, five more slides were taken, which contained 6, 2, 3, 2, 5 microfilariae respectively, i.e., 90 per ml. The results of subsequent examinations are shown in Table II. One hour after the transfusion, the patient began to have an urticarial rash on the limbs and back. On the first day, the patient mostly lay on his bed.

On the second day, he moved about fairly frequently and sat outside the ward for part of the time. He continued to move about fairly frequently during the day-time on the following days. On the 9th day he insisted on being discharged.

These cases, in which microfilariae were transferred to a fresh human subject, may be summarized thus : (*a*) In all cases a great number of the microfilariae quickly disappeared and were not found again ; in two cases all the injected lavae disappeared, and in two cases 90 per cent. disappeared. (*b*) In the two cases in which 10 per cent. of the microfilariae remained, they continued in the blood for about 10 days and for more than 8 days respectively. During this time the microfilariae of the first case showed no definite evidence of periodicity, and the variations in number which occurred at different times may easily have been due to chance. In the second case the results are ambiguous, and it is impossible to decide whether a certain degree of periodicity was or was not present ; if it was present, it was slight.

B.—INJECTION INTO ANIMALS

In these experiments, the microfilariae were separated from suitable human blood by a technique based on one also described by Knott (1935). About 10 ml. of blood were added to 40 ml. of 0·1 per cent. sodium citrate, which laked the blood and prevented clotting. The microfilariae were concentrated by centrifuging and suspended in a mixture of hydrocele fluid and Locke's solution. In later work, heparin was added to the citrate solution and to the Locke-hydrocele fluid in a concentration of about 1 : 10,000, to prevent clotting. After injection of microfilariae in various ways, the animals were killed at suitable periods and the blood and other body fluids were examined. The organs were then fixed in formalin, so that the place and manner in which the microfilariae were destroyed could be studied. If it proves possible later to complete this histological examination, the results will be described in a subsequent note ; so far, the study has been limited to a few samples.

(i) *Microfilariae injected intravenously*. Mice were injected with 0·2–0·3 ml. of the suspension ; the injections often caused severe prostration, and more than 0·3 ml. was usually fatal. The results of three experiments (A, B and C) are shown in Table III. Allowing for irregularities among the different animals, it is seen that most (over 80 per cent.) of the microfilariae disappear from the circulating blood within the first hour, and that the rest diminish progressively, all being gone in about 40 hours. There is no evidence of any diurnal-nocturnal fluctuation in the numbers of microfilariae in the blood-stream, although it must be admitted that the waking and sleeping hours of mice are too irregular to be followed. In some of the mice examined histologically, a few microfilariae were seen in the capillaries of the lungs, of the liver and of the glomeruli of the kidneys, especially in the latter situation ; phagocytosis was not observed. Injections were also made into a guinea-pig and into a monkey.

Four ml. of fluid, containing about 17,000 microfilariae, were injected intracardially at 10.45 a.m. into a guinea-pig weighing 660 gm. ; there should have been about 340 microfilariae per ml. of guinea-pig's blood. At 11.45 a.m. four films from the ear (equal to ? 100 mm.^{3} blood) contained 2 microfilariae, both in their sheaths (? 20 microfilariae per ml.). The guinea-pig was killed at 10.30 p.m., and six films of venous and heart blood (? 200 mm.^{3}) contained 1 microfilaria, which was still ensheathed, but the nuclei seemed granular and stained poorly. In the second case, 5 ml., containing 12,000 microfilariae, were injected intravenously into a monkey weighing 1·1 kilo., which would be

TABLE III

Showing the persistence of microfilariae (*bancrofti*) in the blood of mice after intravenous injection

Experiment A		Experiment B		Experiment C	
Time, in hours	Mf. per ml. blood	Time, in hours	Mf. per ml. blood	Time, in hours	Mf. per ml. blood
Start	5,600*	Start	8,000*	Start	6,300*
Died at once	6,000 ?	Died at once	1,400 ?		
		0·6	780 ?	0·9	344
		1·25	665		
		2·5	400		
		6·7	360		
9	20	10·5	56	8·5	30
15	4				
21	80	23	4	20	8
32	12				
		40	0	40	0
65	0	60	0		
87	0				

*Calculated on the assumption that a 20 gm. mouse has 1·6 ml. blood.

sufficient to produce 140 microfilariae per ml. of the monkey's blood. Four films (? 40 mm.^{3} blood) made from the ear immediately after injection contained no microfilariae. The monkey was killed 24 hours later, and 0·3 ml. of heart blood was spread on slides ; no microfilariae were found.

(ii) *Microfilariae injected intraperitoneally.* Microfilariae were injected intraperitoneally into mice, with the results shown in Table IV. They were found in the peritoneal exudate during the seven following days, but not after 11 or 14 days. They were actively motile, and most of them remained inside their sheaths, although sometimes the back part of the sheath trailed along behind the larva. No definite morphological change was seen

in these larvae ; some of the forms observed after seven and a half days were shorter and thicker than usual, and some were motionless, but this may have been due to pressure by the cover-slip. In the stained preparations, some of

TABLE IV

Showing the survival of microfilariae (*bancrofti*) when injected into the peritoneal cavity of mice

Experiment D				Experiment E			
Time, in days	Findings in peritoneal cavity			Time, in days	Findings in peritoneal cavity		
	Exudate	Microfilariae	W.B.C.		Exudate	Microfilariae	W.B.C.
Start	—	8,500 injected	—	Start	—	10,000 injected	—
0·2 ? (found dead)	much, sticky, brown	++ (cocci ++)	++ poly.++ ; monocytes +				
0·6	scanty, milky	+	++ poly.+ ; monocytes+				
1·4	ditto	++ some outside sheaths	++ mono.++ ; endothelial+				
2·4	ditto	++ active ; all in sheaths	++ mono.++ ; lymph.+				
4·5	ditto	++ (as above)	++ (as above)	4·5	scanty, milky	++active; in sheaths	++
7·5	ditto	7 found	++ lymph.++ ; mono.+				
14	moderate	none	++ lymph.++ ; mono.++	11·5	moderate, milky	none	++ lymph.++ mono.++
17	none	none	+ lymph.++ ; mono.++				

the leucocytes appeared to be applied to the microfilariae, but apparently this was only a terminal phenomenon occurring as the slide dried. In fresh preparations, the microfilariae writhed about and glided through the masses of phagocytes ; and adhesion between the cells and the larvae was rare. Preliminary histological examination of some of these animals shows focal collections of

monocytes in the subperitoneal tissues, but no microfilariae have been seen in the few sections examined to date. The blood of all these mice (about 0·25 ml.) was examined at death, but no microfilariae were ever discovered. Fülleborn (1929) obtained similar results ; he found that *Mf. bancrofti* would live for 10 days in the peritoneum of mice, but did not go over into the general circulation.

(iii) *Microfilariae injected subcutaneously.* In order to study the way in which these larvae are destroyed when thus introduced into an unnatural host, injections were made subcutaneously into the ear of rabbits or into the anterior abdominal wall of rats. After various periods the animal was killed and the part preserved in formalin. About 7,000 microfilariae were injected in about 0·3 ml. of fluid. A small swelling was produced by the injection, but this disappeared in a few hours, after which time there might be slight local redness, or there might be no trace visible to the naked eye. In a few of the rats killed 11–33 hours later, solitary microfilariae were found in scrapings from the under surface of the skin at the site of inoculation, and in sections from a rat killed after 42 hours a few larvae were seen surrounded by monocytes ; but no definite phagocytosis was observed in any of these cases. No microfilariae could be found in large thick films taken from the blood of these animals.

DISCUSSION

Transference of microfilariae into a clean natural host has previously been performed by Murgatroyd (1933), Knott (1935) and Rao (1936) with *Mf. bancrofti* in man, and by Fülleborn (1929), Hinman, Faust and DeBakey (1934) and Augustine and Drinker (1935) with *Mf. immitis* in dogs ; and the subject has been reviewed by Lane (1937). (In addition, Knott (private letter, no further details) transfused microfilariae into one other case : the recipient was observed for five weeks, during which time his blood was positive ; four months later it was found to be negative.) Taking only the 11 published cases in which injections were made into a human subject whose blood was previously negative, it is found that in six cases no microfilariae were found in the blood of the recipient after the first few hours, in two cases (Knott's A.C. and W.C.) a few microfilariae were found after two and two and a half days respectively, and in three cases (Knott's E.D. and cases I and IV) the blood of the donor contained appreciable numbers for several days. In one of these three cases (E.D.), the microfilariae appear to have exhibited periodicity ; in the other two, periodicity could not be demonstrated. In the various transfusions into dogs, microfilariae have often been observed in the blood of the recipient for considerable periods, but periodicity has never been recorded. In all cases (human and canine) over 90 per cent. of the microfilariae injected disappear from the donor's blood in a few hours and are unaccounted for. This disappearance may be assigned to the following causes : (1) Mechanical retention of the micro-

filariae in the capillaries, e.g., in the glomeruli of the mice described above ; note also Knott's cases, where microfilariae were injected into the brachial artery, and the observations of Yorke and Blacklock (1917). (2) Emigration of the microfilariae into the lymphatics and tissues ; shown by Augustine and Drinker (1935) to occur with *Mf. immitis*. (3) Active destruction of the microfilariae by the reticulo-endothelial system or some other defence mechanism. This has been shown by O'Connor to occur in rock-doves with *Mf. columbigallinae* (Lane, 1937) and also with the same microfilaria injected into frogs (Augustine, 1937). It has not yet been clearly demonstrated to occur in man, although it certainly must occur to some extent.

Theoretically the question whether or not the periodicity of microfilariae depends upon the presence of the adult worms should easily be solved by transfusion experiments of the type under discussion ; in *practice* it has proved difficult and inconclusive. Sufficient experience has now accumulated to show that, if enough microfilariae are injected, some of them will be found in the blood of the recipient during subsequent days ; but the only result which can be conclusive is the result in which the microfilariae show unmistakable periodicity in the blood of the recipient, and this result has not been observed, except in the single case (E.D.) recorded by Knott ; all other results can be explained in several contradictory ways. It must be concluded, therefore, that the evidence of transfusion experiments is indecisive ; with a little forcing, the results obtained to date can be made to fit either hypothesis.

It may be that further experiments under more favourable circumstances will lead to more decisive results ; but it must be remembered that it is very difficult to reproduce the exact condition of affairs found in the microfilarial carrier ; probably he possesses defence mechanisms, humoral or cellular, which are poorly developed in the recipient of the experiment. In planning such work, attention should be paid to the following points : (1) the possibility of a latent infection present in the recipient may be diminished by testing his skin-reaction to *Dirofilarial* antigen ; (2) the number of microfilariae injected should exceed one million ; (3) the waking and sleeping hours of the recipient, subsequent to the transfusion, should be carefully regulated.

SUMMARY

1. Microfilariae (*Mf. bancrofti*) were injected intravenously into four human subjects, whose blood had previously been shown to be free from microfilariae.

2. In two cases, subsequent examination of the blood of the recipient failed to demonstrate microfilariae.

3. In the other two cases, about 90 per cent. of the microfilariae injected disappeared quickly, but some persisted in the blood of the recipient and could be demonstrated for nine days and more than eight days respectively. These microfilariae which persisted did not exhibit definite nocturnal periodicity,

such as is characteristic of *Mf. bancrofti.* However, it should be noted that the sleeping and waking hours of these two recipients may have been irregular.

4. These experiments were undertaken in an attempt to decide whether the nocturnal periodicity of *Mf. bancrofti* is dependent upon the presence of the adult worms (as suggested by Lane) or not ; it is considered that the results obtained are not conclusive.

5. Microfilariae (*Mf. bancrofti*) were also injected into mice and other animals. When injected intravenously, they rapidly disappeared from the circulating blood within 20–30 hours ; on histological examination, a few were found in the capillaries of the internal organs.

6. When injected intraperitoneally, the microfilariae persisted, alive and active, for about seven days ; there was no evidence of phagocytosis by the leucocytes of the peritoneal exudate ; they were never found to pass into the circulating blood.

7. When injected subcutaneously, most of the microfilariae disappeared in about 40 hours, but they could not be found in the blood.

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AN UNUSUAL CASE OF LEISHMANIASIS TREATED WITH 4:4′-DIAMIDINO DIPHENOXY PENTANE

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We have recently observed a case of leishmaniasis presenting such a number of interesting features that we have thought it worth while recording them.

The patient, a male of Taishi race, aged 24, from the Blue Nile district, was admitted to Omdurman hospital on November 4th, 1939, complaining of fever of one month's duration. On admission the patient was emaciated, anaemic, and looked a very ill man. He was also asthmatic. Forehead, cheeks and abdomen were deeply pigmented. There was fever ranging to 104° F., with a proportionately rapid pulse, and the spleen was palpable to the umbilicus. Blood: R.B.C. 2,890,000, W.B.C. 1,650; differential count: P. 36 per cent.; L. 62 per cent.; L.M. 1 per cent.; E. 1 per cent. Splenic puncture revealed numerous Leishman-Donovan bodies, but none were found in nasal smears.

He was given a course of neostibosan, beginning with 0·05 gm. Subsequent injections were given every 3rd day, and the dosage was increased with each injection by 0·05 gm. till a maximum of 0·3 gm. was reached, and this latter dosage was continued till a total of 3·35 gm. had been given. The irregular high fever began to subside by lysis after 13 days, when 0·6 gm. had been given. It had subsided completely by the 19th day, after 1·15 gm. had been given, and the patient remained afebrile until his discharge on December 18th (45th day). The spleen was still palpable two fingers' breadth below the costal margin.

He was readmitted 13 days later (on December 31st) complaining of weakness and tingling in both legs for 10 days. Clinical examination revealed a bilateral anterior tibial paresis, with double foot-drop. There was also a very conspicuous nodular depigmented eruption on the face (Plate I, fig. 1), and a minutely punctate rash over the remainder of the body. The nodular depigmented eruption extended to the mucocutaneous junction of the nose, and anterior rhinoscopy revealed a large flat ulcer inside the nose on the nasal septum. Smears from this ulcer and from a nodule excised from the face contained Leishmania. The patient was afebrile, and, apart from the

skin eruption and foot-drop, felt perfectly well. The spleen was palpable three fingers' breadth below the costal margin.

Beyond supporting the feet, no specific treatment was given for the neuritis, which improved rapidly. A further course of neostibosan, which would ordinarily have been given for the skin condition, was withheld, since the neuritis was apparently the result of antimony intolerance. After 49 days in hospital, when the drug became available, the patient was given a course of 4 : 4′-diamidino diphenoxy pentane, an aromatic compound (see Lourie and Yorke, 1939) which one of us (R. K.) had already found effective in two cases of kala-azar. The drug, dissolved in 10 c.cm. of distilled water, was given on alternate days by the intravenous route. Ten doses of 80 mgm. and 13 doses of 100 mgm. were given, a total of 2·1 gm. No toxic symptoms were observed. During this course the nodular eruption on the face disappeared and the pigmentation became normal. The punctate eruption on the rest of the body subsided, leaving small depigmented areas, and the spleen shrank to one finger's breadth. The patient was discharged on May 10th, 1940, apparently cured.

He was seen one month later. The spleen was not palpable. There were still depigmented areas on the trunk, but the skin of the face was normal (Plate I, fig. 2). A thin bluish covering of new epithelium had grown over the ulcerated area in the inside of the nose. Smears made from the skin and from the inside of the nose failed to reveal Leishmania. Asthma had been troublesome since discharge from hospital, but there had been no return of fever.

COMMENTS

The main points of interest in this case-history may be noted briefly :

1. The rapid reaction to antimony treatment observed in this case is unusual in the Sudan, where the action of this drug on the signs and symptoms of the disease is usually slower than in India, and a larger total dosage is generally required for cure.

2. The development of peripheral neuritis, limited to the anterior tibial nerve, is an interesting and unusual complication. Presumably this may be regarded as an unusual toxic effect of antimony. More recently a second instance has been observed by one of us (D. R. M.), in which a kala-azar patient who was treated with neostibosan developed bilateral foot-drop some time after his discharge from hospital as cured.

3. The dermal condition which developed as the visceral disease subsided is of considerable interest. Clinically the nodular eruption on the face resembles very closely some of the conditions described from India under the name of post-kala-azar dermal leishmaniasis (cf. Napier and Das Gupta, 1934), except that in the present case the interval between clinical cure of the visceral disease and the appearance of the skin condition was approximately a month instead of

1–2 years. Attention has been directed elsewhere (Kirk and Sati, 1940) to the development of punctate and papular rashes in cases of Sudan kala-azar which are progressing favourably under treatment. It may be noted that in the present case development of the nodular eruption was associated with a very satisfactory reaction to treatment of the visceral condition.

4. Post-kala-azar dermal leishmaniasis is, as a rule, very resistant to treatment (Napier and Haldar, 1930). The present paper records what appears to be a very satisfactory response to treatment with a new drug. However, it is a little uncertain whether the skin condition in the present case is strictly comparable to the dermal leishmanoids of India. The appearance of minutely punctate rashes during treatment is not uncommon in Sudan kala-azar, and these minor rashes may disappear spontaneously after some months.

5. The concomitant development of dermal leishmaniasis and an ulcerative condition in the nasal cavity is particularly interesting. From time to time, cases of oral leishmaniasis are seen in the Sudan (Christopherson, 1914; Susu, 1917; Humphreys and Mayne, 1935), and some of those correspond very closely with the clinical description of espundia (South American mucocutaneous leishmaniasis). It is at present unknown whether the African condition is due to the parasite of kala-azar or to a separate strain of Leishmania. In most cases no evidence of either cutaneous or visceral infection can be found, but one of us (R. K.) has seen two instances in which visceral and oral leishmaniasis were present concurrently in the same individual. The present case is the first one in which visceral, dermal and mucocutaneous leishmaniasis have been found in one individual. The associated circumstances suggest that these are all part of the same infection, but the clinical picture of the mucocutaneous condition is not that of an established case of espundia, in which intensive destructive lesions are usually present by the time the patient comes for treatment. It cannot be proved in the present case that without treatment the intranasal ulcer would ultimately have produced extensive destruction of the nasopharynx, although it seems reasonable to assume that it might.

Ulceration conditions inside the mouth due to Leishmania have been described from India by Napier and Das Gupta (1934), who think that the lesions are not comparable to the South American espundia, but should be regarded as a variety of post-kala-azar dermal leishmaniasis. In the present case it is of particular interest that an ulcerative condition inside the nose appeared simultaneously with a skin eruption very closely resembling post-kala-azar dermal leishmaniasis.

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FIG. 1

FIG. 2

FIG. 1. Post-kala-azar nodular depigmented eruption; before treatment. Leishman-Donovan bodies +.

FIG. 2. After treatment. No Leishman-Donovan bodies in skin-scrapings.

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OBSERVATIONS ON THE BIOLOGY OF *PLASMODIUM GALLINACEUM* BRUMPT, 1935, IN THE DOMESTIC FOWL, WITH SPECIAL REFERENCE TO THE PRODUCTION OF GAMETOCYTES AND THEIR DEVELOPMENT IN *AËDES AEGYPTI* (L.)

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INTRODUCTION

The convenience of *Plasmodium gallinaceum* as experimental material has recommended it to a number of workers, and, since its original description by Brumpt in 1935, a considerable literature relating to it has accumulated. Brumpt himself (1936) has investigated the susceptibility of a number of different species of birds to the parasite, and has shown that the latter may be transmitted by *Aëdes aegypti* (L.) and *A. albopictus* (Skuse). Roubaud *et al.* (1939) record complete development also in the non-tropical species *A. geniculatus* (Olivier). Information on the length of the prepatent period and on the course of the infection in the fowl is given by Brumpt (1936), Brumpt *et al.* (1937), Henry (1939) and Jacobi (1939). Giovannola (1938) has investigated the periodicity of the asexual cycle. Much attention has also been devoted to the exo-erythrocytic forms, but these have been neglected in the present work, as gametocytes, so far as is known, occur only in erythrocytes or cells of that series. The gametocyte-incidence in malaria in canaries caused by *Plasmodium* spp. has been investigated in detail by Huff (1927), Shah (1934) and, in particular, by Gambrell (1937). The results of the work of these authors will be compared with our own findings in the case of *P. gallinaceum*.

We are indebted to Professor R. M. Gordon, at whose suggestion the work was undertaken, and to Dr. D. R. Seaton and Mr. D. Dagnall, who have helped considerably with the counts and mosquito dissections.

MATERIAL AND METHODS

The strain of *P. gallinaceum* used was originally obtained from Dr. W. Schulemann of the University of Bonn, and has been maintained since 1938 in

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domestic fowls, being transmitted, with one recent exception, entirely by blood inoculation. Since its reception in the Liverpool School of Tropical Medicine, it has gone through about 30 passages.

Owing to war conditions, considerable difficulty was experienced in obtaining fowls of standard breed and age. Those used were White Leghorn, Rhode Island Red and Light Sussex, either pure or cross-bred, and mainly 12–16 weeks old. Blood for counts of various kinds was at first withdrawn from the leg- or wing-vein, but these methods were later discarded, as such blood, after clotting has taken place in the vein, is much diluted with serum. Comb-blood, likely to be less variable in this respect, was afterwards exclusively used. Parasite counts were made in thin films stained with Giemsa or Leishman stain. The total parasite count is expressed as the number of parasites per 1,000 erythrocytes, and usually such a number of cells was counted that the probable error would be about 10 per cent. of the observed number of parasites. The requisite number of cells was determined from the formula of Hartman (1927). At the beginning and end of the infection, when there were less than 40 parasites per 1,000 red cells, examination of the required number of cells would have occupied a time disproportionate to the importance of the count, so that values recorded below this level are subject to a larger probable error. To counteract inequalities in the distribution of the parasites in the film, the ordinary precautions were observed. In making counts of the proportion of each stage of the parasite present, 200 parasites were counted whenever possible. Again, when there were less than 40 parasites per 1,000 erythrocytes, the counts are based on smaller numbers. The asexual parasites were divided into two classes, in contrast to the four classes of Giovannola (1938). Asexual parasites were called either trophozoites or schizonts, and all which satisfied the following conditions were placed in the schizont category : (1) chromatin divided clearly into two or more masses ; (2) pigment clumped ; (3) parasite larger than half the longest diameter of the containing red cell. That is, the definition of a schizont as given by Wenyon (1926) was narrowed down to include only those forms which were indubitably going on to schizogony, no attempt being made to distinguish young schizonts from pre-gametocytes. Sexual forms were included in the differential counts, but no attempt was made to distinguish male and female gametocytes, which were only noted when fully developed.

The number of gametocytes per c.mm. of blood was estimated by a method which was in substance that described by Christophers, Sinton and Covell (1939). A rabbit-corpuscle suspension was used, and, as the rapidity of clotting of the fowl blood rendered thorough mixing difficult, the suspension was made in isotonic citrated saline of the constitution mentioned below, instead of in normal saline. The suspension contained about 200,000 rabbit erythrocytes per c.mm. (mean of 10 counts, 201,000). Five hundred rabbit cells were counted in films made from a mixture of equal volumes of the suspension and of fowl blood. The number of gametocytes seen during this examination, therefore, represented

those present in 0·0025 c.mm. of fowl blood. This method, which estimates directly the number of gametocytes in a given volume of blood, was considered preferable to that employed by Huff (1927), who evaluated the gametocytes indirectly by parasite and blood-cell counts. It should be noted, however, that the erythrocytes, to which the total parasite counts in the fowl blood are related, may fall as low as 1·5 million per c.mm. at the peak of the infection (Jacobi, 1939).

Fowls were infected both by subinoculation and by means of mosquitoes. In the former case 0·5 c.cm. of a 1 : 10 dilution of infected blood in isotonic citrated saline (0·7 per cent. sodium chloride, 0·5 per cent. sodium citrate) was injected intravenously. The approximate number of parasites inoculated could then be calculated from the cell and parasite counts of the blood used. Fowls whose infection by means of the insect vector was desired were exposed to female *Aëdes aegypti* 14 or 15 days after the latter had fed on an infective fowl. As far as possible, single mosquitoes were used to infect, the glands being examined immediately after the insect had engorged.

The mosquitoes used were exclusively *Aëdes aegypti* (L.) of a strain which was probably originally obtained from Africa and has been maintained since 1938 in the insectarium described by Bertram and Gordon (1939), the adult females being fed on rabbits. Larvae were raised from the eggs of stock adults by the methods described by Tate and Vincent (1936) for the rearing of autogenous *Culex pipiens* L. Pupae were transferred daily to net-covered containers of the type described by these authors, and the adult females were subsequently removed every 24 hours. These were set aside, in lots of about 150, in net-ended glass cylinders about 7 cm. in diameter and 10 cm. long. All mosquitoes, except when being manipulated, were kept in darkness in the insectarium (see Bertram and Gordon, 1939) at a temperature of 24° C. and a relative humidity of about 80 per cent. They were allowed access to 40 per cent. cane-sugar solution, except for a period of four days before being offered a blood meal, when they were completely starved in order that they would feed more readily.

When it was desired to feed mosquitoes on fowls, the birds were trussed by the method recommended by Dunn (1932), and a net-ended glass cylinder containing the mosquitoes was applied to the breast and was held in position by elastic bands and tapes. The feeding operation was carried out in a laboratory in which the ordinary room-conditions of temperature and humidity prevailed, but during application to the fowls the cylinders were kept covered with black cloths and were warmed by means of a bench-lamp at a distance of about 20 cm. Female *A. aegypti*, starved completely for the previous four days, usually fed readily under these conditions, but those which did not were generally induced to gorge by the application of saliva to the skin of the fowl.

Batches of about 20 female *Aëdes aegypti*—derived by random selection from the lots of 150 adult females mentioned above—were fed on the fowls at

frequent intervals during the apparent infection. The mosquitoes were about 6 days old when given this blood-meal. They were dissected 10 days later and the numbers of oöcysts present on the mid-gut wall were counted. An average number of oöcysts was computed for each batch. Batches of *A. aegypti* as large as were available were used in all instances, but sometimes the number of gorged mosquitoes obtained was limited by their reluctance to feed, and, further, all did not survive the 10-day period before dissection. The average number of mosquitoes surviving until dissection on the 10th day was 11 ; in only one case was the number less than 5.

In the experiments the average number of oöcysts developing in the mid-guts of a given batch of *Aëdes aegypti* was taken as an indication of the number of gametocytes, capable of further development in the mosquito, which were present in the peripheral blood of the fowl at the time of feeding. The reliability of such counts may be questioned on the ground of the existence of individual immunity to infection on the part of the mosquito. Huff has devoted attention to the study of immunity to malarial infections in *Culex pipiens* L. References to all his papers may be found in the summary of knowledge of immunity in invertebrates by the same author (1940). The salient points which he discovered are :

1. Individual immunity to infection by *Plasmodium cathemerium* Hartman and *P. relictum* Grassi and Feletti exists in *Culex pipiens* L. and is a matter of degree : in a batch of mosquitoes, all fed on blood containing large numbers of gametocytes, some became heavily, others lightly, infected, while the remainder did not become infected at all. As all of the batch were kept under identical conditions of temperature and humidity, these factors were presumed to have had no influence in determining these differences.

2. The character of insusceptibility behaves as a Mendelian dominant.

3. Mosquitoes which did not become infected by one meal were also immune to a second, and, in a series of susceptible mosquitoes which were allowed to feed twice on heavily-infected birds (gametocyte intake, 53,200–266,000 per meal), there was a marked correlation between the number of parasites arising in individual mosquitoes from these two meals.

The strains of *Culex pipiens* used by Huff were stenogamous and therefore probably autogenous also. Roubaud (1933), after a few experiments with an autogenous Parisian strain of *C. pipiens*, concluded provisionally that it was only slightly susceptible to infection with *Plasmodium relictum*. The same author with Mezger (1934) later compared the susceptibility of one autogenous and two anautogenous races : the former he found to become infected in all cases, but the latter only in about 40 per cent. of cases (*P. relictum*). These generally conflicting results are probably to be explained by the findings of Tate and Vincent (1934) that autogenous and anautogenous races of *C. pipiens* behave similarly towards the same strain of *P. relictum*, but that different infection-rates and intensities were produced by different strains of that parasite. If a condition of individual

immunity existed in *Aëdes aegypti* for infection with *Plasmodium gallinaceum*, it is obvious that the value of oöcyst counts for our purpose would be much reduced, as it would be necessary to employ large batches of mosquitoes to ensure that the proportion of immune individuals would be comparatively constant. Further, the last finding of Huff indicates that the number of oöcysts developing in the stomach of *C. pipiens* is determined not entirely by the number of gametocytes ingested, but to some extent by the physiological make-up of individual mosquitoes. The results of Brumpt (1936), who obtained 100 per cent. infection in 35 *Aëdes aegypti* after they had been fed on fowls infected with

TABLE I

Showing percentages of negative mid-guts in 73 batches of *Aëdes aegypti* fed on untreated infective fowls ; arranged according to average oöcyst count in individuals which became infected

Average oöcyst count in mosquitoes which became infected	No. of batches	Total nos. of mosquitoes in batches	Total no. of mosquitoes which showed no oöcysts	Percentage of mosquitoes which showed no oöcysts
1–10	23	312	91	29
11–20	24	373	70	19
21–30	9	109	6	5·5
31–40	7	108	5	4·6
41–50	4	47	1	2·1
51–60	1	17	0	0
61–70	4	53	0	0
71–80	1	16	0	0
Totals	73	1,035		

P. gallinaceum, seemed to show that that species was wholly susceptible. We give in Table I data relating to 73 batches of *A. aegypti*, each exceeding 10 in number, which were fed on fowls whose infectivity was shown by the occurrence of oöcysts on the mid-guts of some of the batch. The batches are arranged in groups according to the average oöcyst count in those mosquitoes which became infected. It is unreasonable to suppose that the number of oöcysts developing on the mid-gut is unaffected by *large* variations in the gametocyte intake : Huff's results are derived from mosquitoes which all took massive numbers of gametocytes.

If a high oöcyst count among individuals known to be susceptible is taken as indicative of a high gametocyte intake, it is evident that when all the mosquitoes took in large numbers of gametocytes all became infected ; no negative mid-guts are recorded when the average oöcyst count exceeds 50 per stomach. If individual immunity occurred it was broken down when the numbers of gametocytes ingested were large. These results closely resemble those of Shah, Rozeboom and Rosario (1934) with *P. cathemerium* and *Culex pipiens*. The conclusion

is, therefore, that with increasing numbers of ingested gametocytes not only does the average oöcyst count in infected mosquitoes rise, but also the proportion of mosquitoes which becomes infected increases, so that the average oöcyst count of a batch of *A. aegypti* (including negative mid-guts) is a reliable indication of the number of gametocytes, capable of further development in the mosquito, in the peripheral blood of the fowl at the time of feeding.

The number of oöcysts developing in the mosquito must, presumably, be influenced by variations in the volume of blood and therefore of the number of gametocytes ingested. This factor was eliminated, as far as possible, by taking only mosquitoes which had fully gorged with blood. The average weight of blood ingested by a female *Aëdes aegypti* is 1·6 mgm. (Neumann, 1909), which is equal to about 1·5 c.mm.

The results of all counts are plotted together for individual fowls. The percentage-form counts and the absolute gametocyte counts are plotted arithmetically. A logarithmic ordinate is used for plotting total parasite and average oöcysts counts, both for convenience in expressing the large ranges found and to express correctly the rate of change of the intensity of the parasitization.

BIOLOGY OF *PLASMODIUM GALLINACEUM*

Prepatent Period

It is well known that the length of the prepatent period of bird malaria may vary widely. Boyd (1925) records prepatent periods varying from 1 to 11 days in 30 canaries infected with *Plasmodium praecox* (? = *P. relictum*) by intramuscular injection.

Similar variations are noted by Brumpt (1936) for *P. gallinaceum* in fowls infected by the intraperitoneal route. In the 40 fowls of widely varying ages in which his table shows no disturbing factors, the prepatent period varied from 4 to 12 days, with a mean of 8·1 days. Jacobi (1939) states that parasites appear in the blood 5–9 days after intramuscular inoculation. With regard to the prepatent period after mosquito-transmission, Brumpt (1936) records exact data of five chickens infected by *Aëdes aegypti*. The prepatent period varied from 5 to 10 days with a mean of 7·0 days. Henry (1939) has investigated the prepatent period in two fowls (500–700 gm. in weight, probably about 6 weeks old) infected by the bite of *Aëdes albopictus*. Parasites were not detectable microscopically in the peripheral blood until 5 and 6 days respectively after inoculation. In the latter case the blood became infective by subinoculation one day before the appearance of the parasites.

In the results which follow, the end of the prepatent period may be defined as the first occasion on which parasites were detected in thin films of the peripheral blood. Though no standard duration of search was observed, a film would not be recorded as containing no parasites unless examined for at least five minutes.

The records of the transmission of *P. gallinaceum*, since it was received

in the Liverpool School of Tropical Medicine*, contain 29 inoculations by the intramuscular route into fowls of a uniform age of 5–6 weeks. The distribution of the prepatent periods relating to these fowls is shown in Table II. The mean prepatent period was 6·41 ± ·34 days (standard error). Records of 16 intravenous inoculations into fowls of the same age were also extracted from the same source. In these birds (Table II) the mean prepatent period was 2·75 ± ·19 days (standard error). The difference in the mean prepatent period in these two groups is certainly significant, $\frac{d}{\sigma_d} = 7{\cdot}6$. The inocula in both these groups

TABLE II

Showing distribution of lengths of prepatent periods after intramuscular and intravenous inoculation

Prepatent period (days)											
1	2	3	4	5	6	7	8	9	10	11	
	+	+	+	+	+	+	+	+	+		
			+	+	+	+	+		+		*Intramuscular*
				+	+	+	+				
				+	+	+	+				Mean 6·41 ± ·34 days
				+		+					
						+					
						+					
						+					
						+					
	+	+	+								
	+	+	+								*Intravenous*
	+	+	+								
	+	+									Mean 2·75 ± ·19 days
	+	+									
	+	+									
	+										

were unmeasured, but were all made from heavily infected donors, so that it is probable that all contained large numbers of parasites. It is therefore reasonable to suppose that this difference is due to the different routes of inoculation.

Table III summarizes the data relating to 13 fowls infected by the intravenous injection of a measured inoculum.

There seems to be some tendency for longer prepatent periods to be associated with infection by small inocula, but it is possible that other factors, e.g.,

*We are indebted to Dr. E. M. Lourie for some of these data, which are derived from controls to experiments performed by him for other purposes.

the age of the host at the time of infection, may influence the length of the prepatent period. Further, though the mean prepatent period in this group of fowls—6·69 ± ·63 days (standard error)—is significantly longer than that recorded above for fowls 5–6 weeks old, it is probable that in all cases the younger fowls also received larger inocula.

In our experience the prepatent period is much more uniform after mosquito-transmission than in the series recorded by Brumpt. Of a total of 16 fowls, 8–16 weeks old, infected by mosquitoes, it only varied between 8 and 11 days, with a mean of 9·0 ± ·15 days (standard error).

The prepatent period following the infection of fowls 5–6 weeks old by the intravenous inoculation of large numbers of parasites is short, most infections

TABLE III

Showing data relating to 13 fowls infected by the intravenous injection of a measured inoculum

Approximate no. of parasites injected	Age of fowl, in weeks	Prepatent period, in days
50,000	12	9
50,000	16	9
50,000	16	9
50,000	12	7
100,000	16	9
500,000	20	9
30 million	16	4
30 „	16	4
35 „	24	6
45 „	16	7
45 „	16	7
70 „	8	5
70 „	8	2

becoming apparent on the second and third days after inoculation. This probably indicates that under these conditions the amount of destruction of the parasites by the host was small, and that the increase in their number caused by the first one or two periods of reproduction was sufficient to allow them to be detected in thin films of the peripheral blood. As the period of schizogony cycle is about 36 hours, the second period of reproduction after inoculation must occur at or before 72 hours after inoculation. With regard to intra-muscular inoculation, Boyd (1925) has shown with *P. praecox* (? = *P. relictum*) in canaries that the smaller the number of parasites introduced, the longer becomes the prepatent period. Fewer than 1,000 parasites failed to produce infection. Hegner (1929) suggests that these results indicate that a proportion of the parasites was destroyed in the tissues by phagocytosis or other agencies, and that in small inocula all the parasites met this fate. Probably the longer

prepatent period which we have demonstrated to follow intramuscular inoculation, as compared with that following intravenous inoculation, when other conditions were fairly standard, is to be explained on similar grounds—that a greater proportion of the parasites is destroyed in the tissues than is the case when they are introduced directly into the blood-stream. With regard to fowls infected by mosquitoes, we have demonstrated that, even in fowls of fairly widely varying age, the prepatent period is very uniform—the coefficient of variation is only about 7. This is considered to indicate that, before the blood is invaded, a preliminary stage is passed through, which requires for its completion a fairly definite space of time. That this stage probably occurs in the reticulo-endothelial system is shown by the work of James (1939), who found exo-erythrocytic forms of *P. gallinaceum* in all of 19 sporozoite-infected fowls killed on the first three days of the apparent infection, while none was detected in 14 subinoculated fowls killed at corresponding times.

Huff (1927) has attached taxonomic importance to the mean prepatent period in three species of canary malaria, but he does not state the route of inoculation. In the absence of this datum, comparison of prepatent periods for different species would appear to be unreliable.

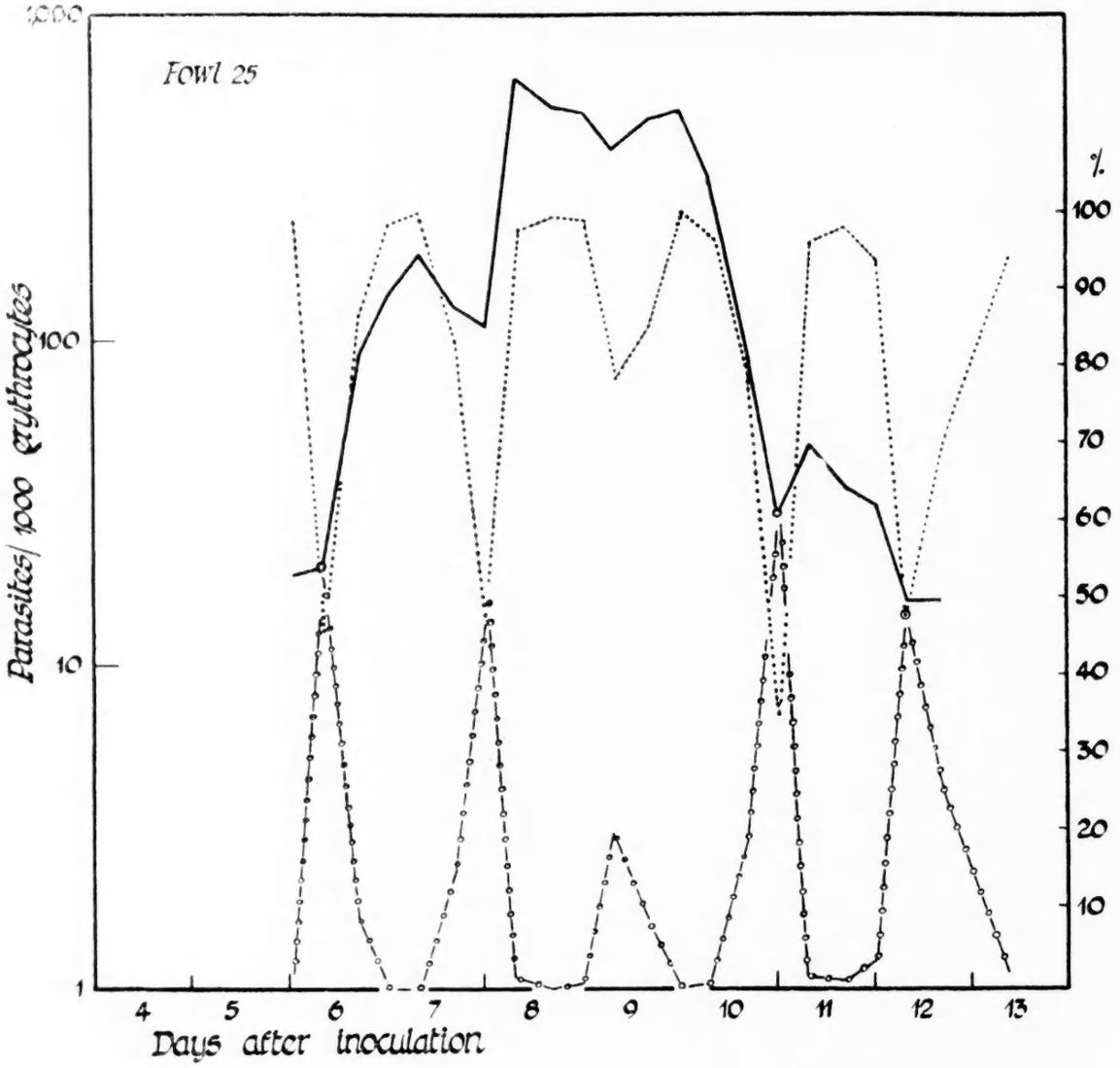
Course of the Infection

Brumpt (1936) gives a table of passages showing a patent period, after subinoculation, varying from 5 to 16 days (mean 10·4). He states that 80–90 per cent. of the erythrocytes may be parasitized. Jacobi (1939), using the same method of infection, states that most fowls show more than one parasite per field ($\times$450) between the 9th and 17th days, but adds that in a few fowls the infection may last longer. Jacobi followed the course of the infection by counts of films made once per day. Giovannola (1938) has made more frequent counts —every six hours—but he studied only the relative proportions of the different forms and did not at the same time follow the intensity of the infection. In the present study, films were made every eight hours (at 1 a.m., 9 a.m. and 5 p.m.) throughout the infections, after both intravenous inoculation of infected blood and mosquito-transmission.

Figs. 1–4 show typical infections progressing to recovery. Fig. 5 gives an example of an infection which caused the death of the host. The first infection (fig. 1) was induced by the intravenous inoculation of infected blood ; the others were transmitted by mosquitoes. As no obvious differences were noted, however, in the course of the apparent infections following these different methods of transmission, they will be discussed together. Since the graphs are sufficiently accurate records of the data, we have omitted, for the sake of saving space, the actual numerical findings from which they were constructed.

In infections progressing to recovery, the duration of the acute attack, counted from the time at which parasites became easy to find in thin films (i.e.,

about 20 parasites per 1,000 erythrocytes) till they again reached this level, varied from 3 to 11 days, but most lasted 4–6 days. The largest number of parasites per 1,000 erythrocytes was reached about the middle of this period (figs. 1–4). There was no noticeable difference in the progress of the infection in fowls which subsequently succumbed to the blood infection, until a corresponding peak had been passed. At that time in such cases an arrest in the fall occurred, and then the number of parasites rose slowly and irregularly until



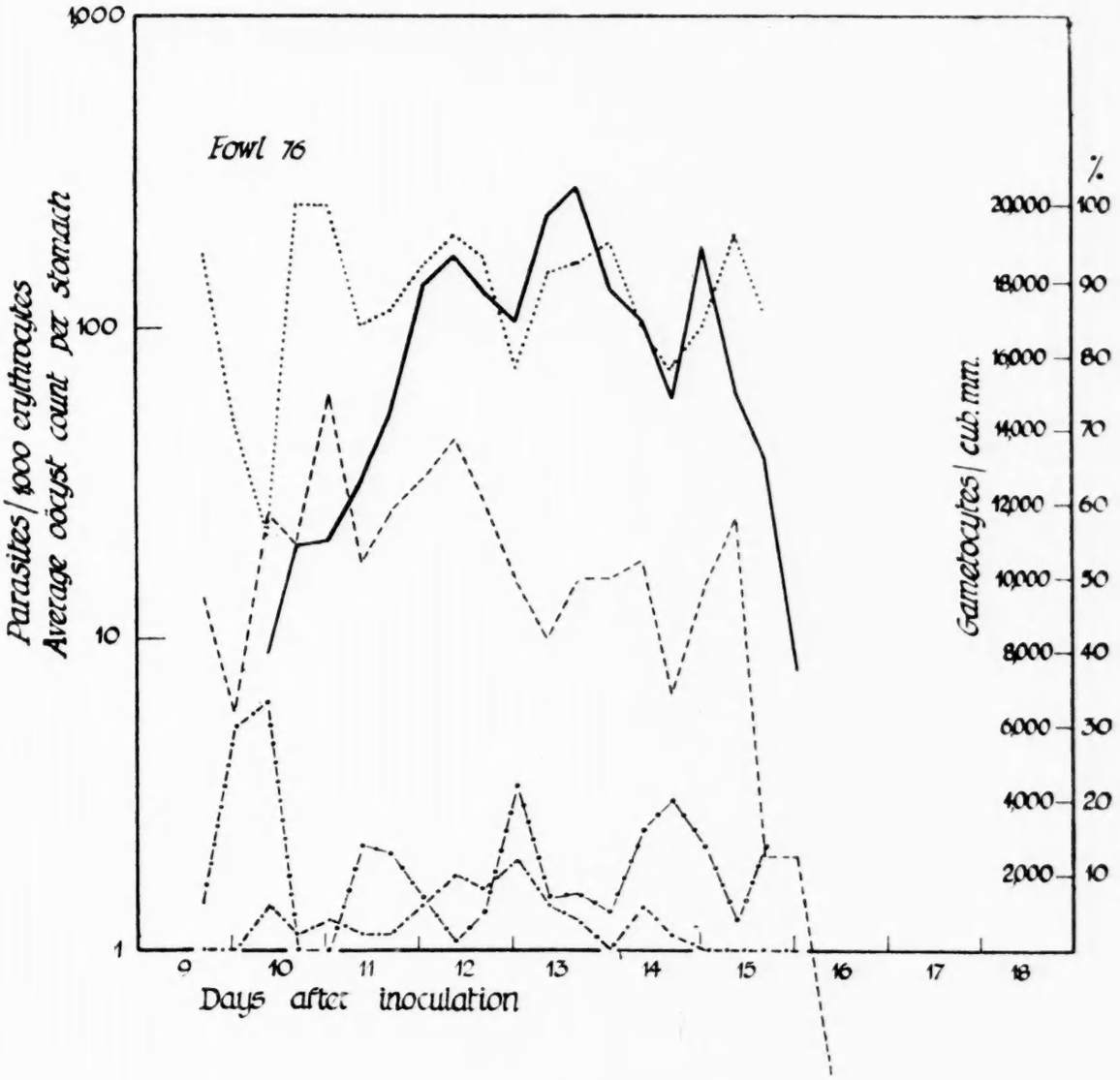
FOWL 25 : AGE AT INOCULATION (INTRAMUSCULAR) ABOUT 16 WEEKS

FIG. 1. Graphs showing total parasite count (logarithmic ordinate) and percentages of trophozoites and schizonts (arithmetic ordinates) in fowl 25 during infection with *P. gallinaceum*.

——— denotes parasites per 1,000 erythrocytes.
..... ,, percentage of trophozoites.
o–o–o–o ,, percentage of schizonts.

death took place when the parasites numbered over 1,000 per 1,000 erythrocytes (fig. 5).

The curve showing changes in the total number of parasites per 1,000 erythrocytes (total parasite curve) shows the expected relationship to the changes in the percentage of schizonts. For purposes of description, the course of the

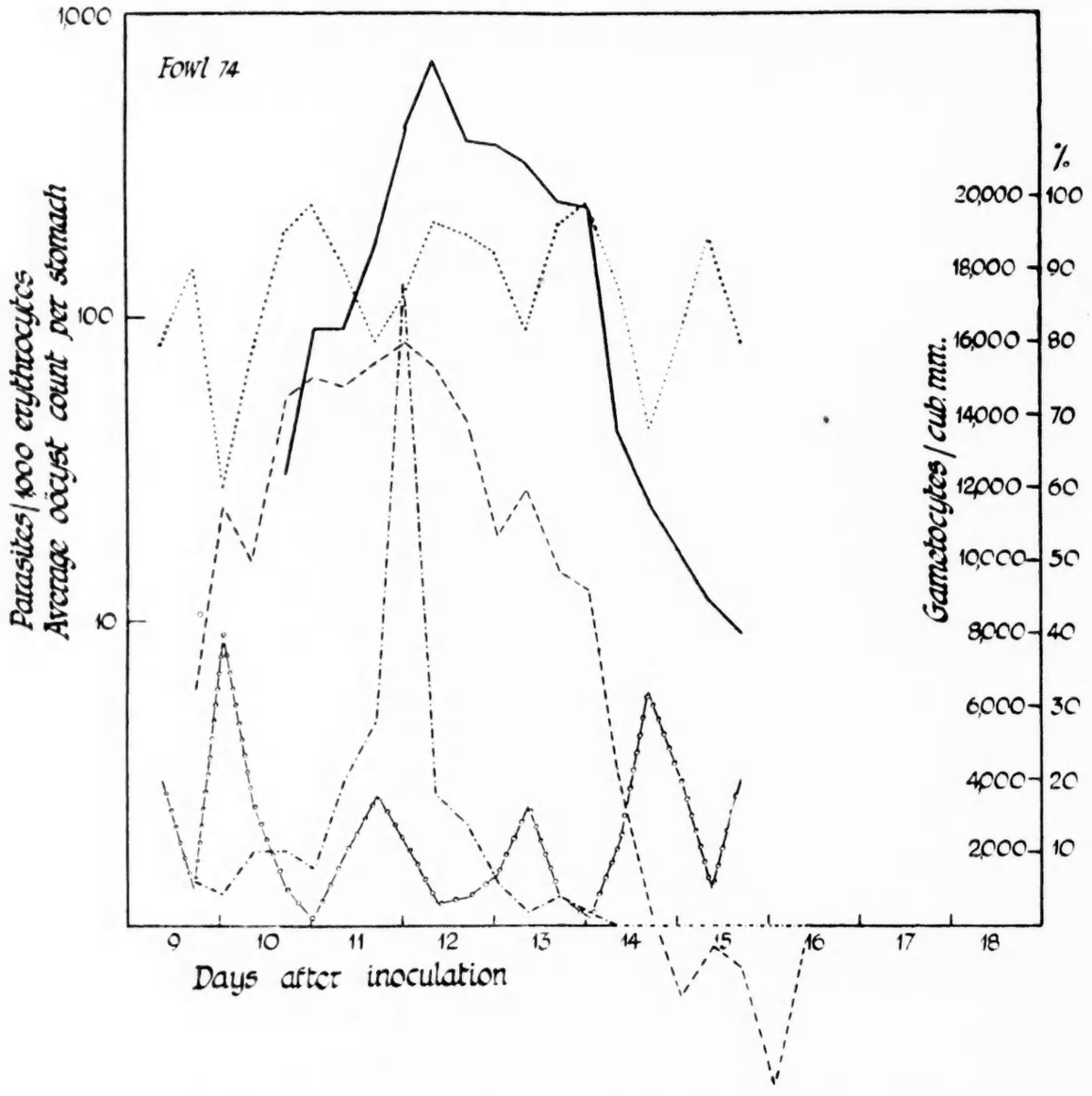


FOWL 76 : AGE AT INOCULATION (BY MOSQUITO) ABOUT 12 WEEKS

FIG. 2. Graphs showing total parasite count, average oöcyst count (logarithmic ordinates), gametocyte count and percentages of trophozoites and schizonts (arithmetic ordinates) in fowl 76 during infection with *P. gallinaceum*.

——— denotes parasites per 1,000 erythrocytes.
– – – – ,, average oöcyst count per stomach.
····· ,, percentage of trophozoites.
o–o–o–o ,, percentage of schizonts.
•–•–•–• ,, gametocytes per c.mm.

infection may be said to be made up of alternating periods of high and low reproductive activity. The former, during which most of the mature schizonts undergo segmentation, corresponds generally with the fall of the schizont percentage curve from its maxima (figs. 1–4). The periods of low reproductive

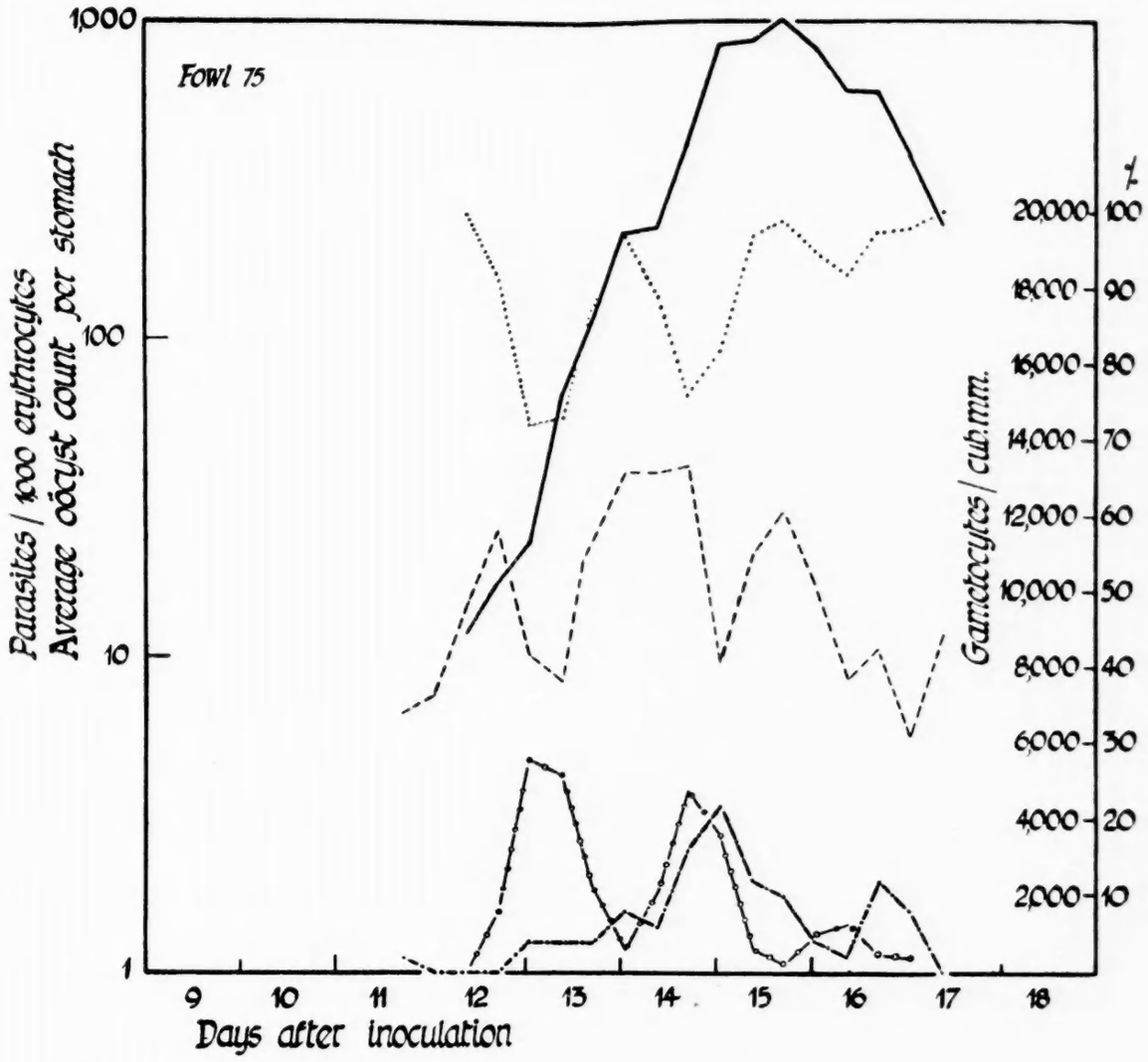


FOWL 74 : AGE AT INOCULATION (BY MOSQUITO) ABOUT 12 WEEKS

FIG. 3. Graphs showing total parasite count, average oöcyst count (logarithmic ordinates), gametocyte count and percentages of trophozoites and schizonts (arithmetic ordinates) in fowl 74 during infection with *P. gallinaceum*.

——— denotes parasites per 1,000 erythrocytes.
– – – – „ average oöcyst count per stomach.
····· „ percentage of trophozoites.
o–o–o–o „ percentage of schizonts.
·–·–·–· „ gametocytes per c.mm.

activity, alternating with these, correspond roughly with the rising of the schizont percentage curve from its minima (figs. 1–4). Most of the schizonts of the previous brood will have segmented before this period, while few of those of the succeeding brood will be mature by its end. It must be recognized, however, that this division into two phases is not a hard and fast one, but it will serve to correlate the total parasite curve with the stage of the asexual

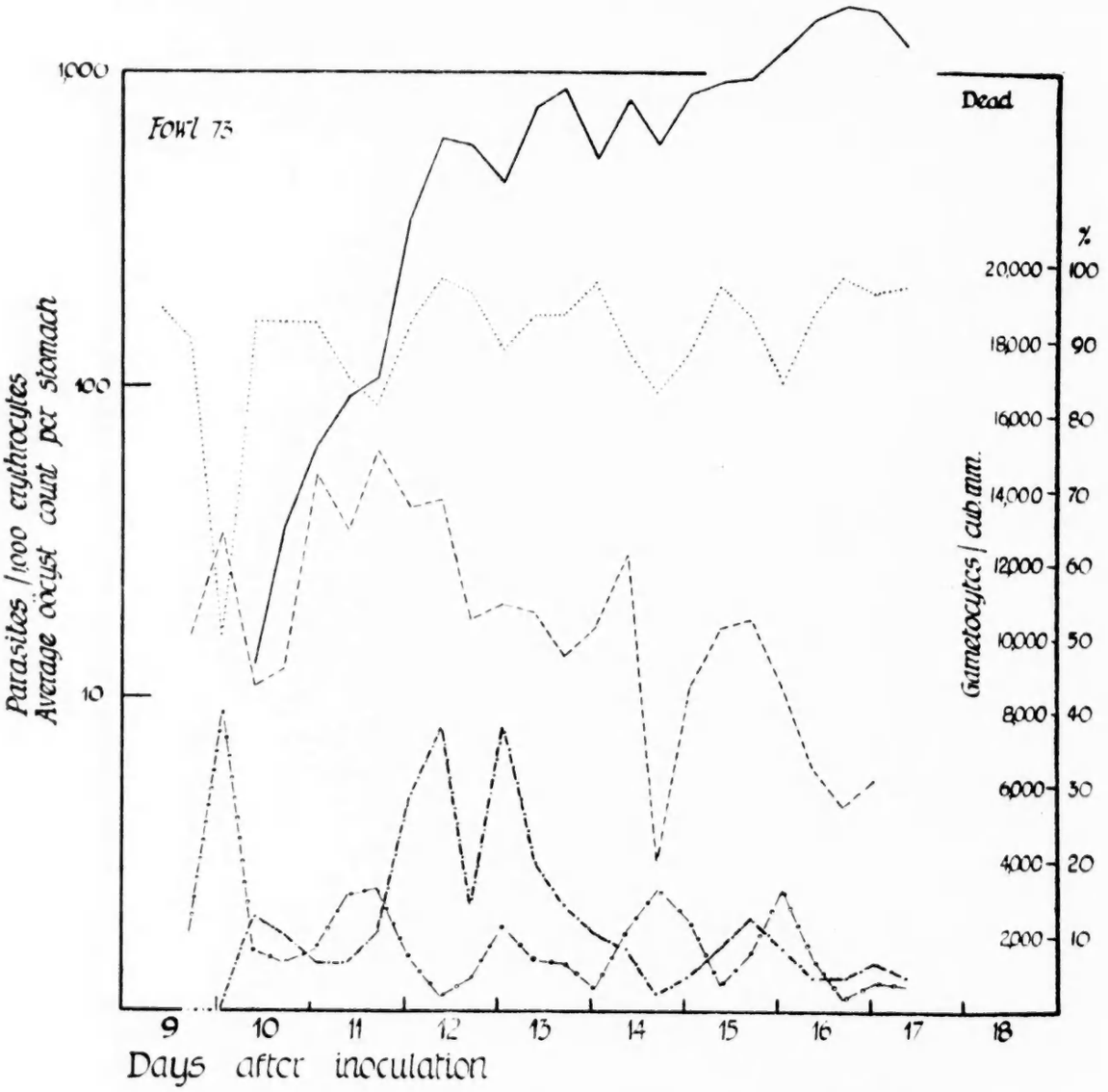


FOWL 75 : AGE AT INOCULATION (BY MOSQUITO) ABOUT 12 WEEKS

FIG. 4. Graphs showing total parasite count, average oöcyst count (logarithmic ordinates), gametocyte count and percentages of trophozoites and schizonts (arithmetic ordinates) in fowl 75 during infection with *P. gallinaceum*.

——— denotes parasites per 1,000 erythrocytes.
– – – – „ average oöcyst count per stomach.
..... „ percentage of trophozoites.
o–o–o–o „ percentage of schizonts.
·–·–·–· „ gametocytes per c.mm.

cycle. In the first half of infections progressing to recovery (figs. 1–4) before the total parasite curve has reached its peak, the phases of high reproductive activity correspond with rapid rises in the total parasite curve. In the second half, when the total parasite curve, as a whole, is falling, the same phases correspond to a transient rise or to a slowing of the rate of fall. During the phases



FOWL 73 : AGE AT INOCULATION (BY MOSQUITO) ABOUT 12 WEEKS

FIG. 5. Graphs showing total parasite count, average oöcyst count (logarithmic ordinates), gametocyte count and percentages of trophozoites and schizonts (arithmetic ordinates) in fowl 73 during infection with *P. gallinaceum*.

——— denotes parasites per 1,000 erythrocytes.
– – – – ,, average oöcyst count per stomach.
..... ,, percentage of trophozoites.
o–o–o–o ,, percentage of schizonts.
·–·–·–· ,, gametocytes per c.mm.

of low reproductive activity a fall in the total parasite count takes place, except early in the infections, when merely a transient slowing of the speed of rise is to be noted (figs. 1–4). These results correspond to those obtained by other workers with *Plasmodium* spp. in canaries. The falls in the total parasite count during the phases of low reproductive activity are comparable with the constant mortality of parasites which Hartman (1927) has shown to take place in *P. praecox* (? = *P. relictum*) between the completion of one period of reproduction and the initiation of the next. The effect, however, is not so clearly defined as in *P. praecox*, which shows a high degree of synchronization and completes schizogony in the short space of about three hours. In *P. gallinaceum* there are usually a few mature schizonts in the blood between the actual periods of greatest schizogony, so that even at those times some merozoites are being added to the population. Further, at the much higher levels of parasitization reached by *P. gallinaceum*, the factors of blood-cell destruction and replacement must be of greater importance and will affect to a greater extent the validity of counts of parasites correlated with the erythrocytes. The effects are, perhaps on this last account, most clearly seen in an infection which did not reach a very high level, e.g., in fowl 76 (fig. 2) which reached a level of only 284 parasites per 1,000 erythrocytes. There was no obvious difference in the intensities of infections differently induced, i.e., by subinoculation or by mosquitoes. Four fowls younger than 12 weeks old at the time of infection all died with massive blood infections of over 1,000 parasites per 1,000 erythrocytes. Of 14 fowls 12 weeks old and more, only one died during the apparent infection. In the 13 recovering infections, the intensity of the infection, as measured by the maximum number of parasites per 1,000 erythrocytes, tended to be less the older the fowl.

Brumpt (1936) records some failures to infect by subinoculation, but these occurred mostly in old birds. We, using fowls all less than 24 weeks old, have never failed to infect in this manner. In a few cases fowls did not become infected after single mosquitoes, whose glands were found on dissection to contain sporozoites, had become engorged on them.

Periodicity in the Asexual Cycle

Giovannola (1938) has already investigated the periodicity of the schizogony cycle of *P. gallinaceum*. By means of differential counts performed every six hours, he determined the periodicity to be about 36 hours and the periods of maximum schizogony to occur alternately at midday and midnight. We have confirmed and extended his observations to show the relation of the asexual periodicity to the level of parasitization. The latter has already been discussed. The maximum proportion of schizonts usually occurs a few hours before midnight or midday (see fig. 1–5), but this difference is to be ascribed to our method of defining a schizont (see p. 136), which included some forms younger than Giovannola's ' forms in sporulation ' category.

In five fowls in which acute infections progressing to recovery were closely followed, $2\frac{1}{2}$–4 schizogony cycles were passed through. In two of four fowls similarly examined, in which the acute blood infection ended in death, the asexual periodicity was maintained unaltered in rhythm till death took place. One of these (fowl 73) is shown in fig. 5. In the other two fowls, however, the well-marked periodicity in the asexual cycle disappeared towards the end of the infection. The proportion of schizonts remained irregularly in the neighbourhood of 10 per cent., while the total parasite count rose slowly until the bird succumbed.

The degree of synchronization of reproduction of the parasite, as judged by the maximum percentage of schizonts occurring, was generally higher in subinoculated fowls than in those infected by mosquitoes. In the former 50–60 per cent. was frequently attained, but in the latter 30 per cent. was rarely exceeded. This difference is brought out by a comparison of fig. 1 with figs. 2–5. Our results with fowls infected by subinoculation agree with those of Giovannola, who also used this method of infection. There seemed to be a tendency in mosquito-transmitted infections for the synchronization to become less marked as the infection proceeded (figs. 2–4). There was no sign of such a change, however, in infections produced by the inoculation of infected blood (fig. 1).

It is worthy of note that the periods of maximum schizogony occur at definite times after mosquito-infection. In 11 of 12 fowls infected by this method, the infection was apparent on the 11th day after mosquitoes had bitten the fowls. In all these cases, three of which (fowls 76, 74 and 73) are shown in figs. 2, 3 and 5, a period of maximum schizogony fell at midnight on day 11–12, and in the remaining example (fowl 75; see fig. 4), in which the infection was slightly retarded in its appearence, a similar period occurred at midday on day 13, i.e., 36 hours after that in the other fowls and in step with the succeeding period in their case. These fowls were infected, mostly by single mosquitoes, within a few hours of midday. After intravenous inoculation of infected blood, on the other hand, no such regularity was observed. For a given day—say the 10th after inoculation—there are three theoretical possibilities: schizogony may take place about midnight on day 9–10, about midday on day 10, or about midnight on day 10–11. The schizogony cycle was followed in three fowls after intravenous inoculation, and in all the infection was apparent on the 10th day. One fowl fulfilled each of these three possibilities; in the infection of fowl 25 (fig. 1) schizogony took place about midnight on day 10–11. The fowl in which schizogony took place at midnight on day 9–10 was infected at the same time and with the same blood as fowl 25, i.e., a citrated suspension of infected blood injected in equal quantities intravenously into two fresh hosts produced infections of which the periodicities were 24 hours out of step.

Study of the conclusions of workers who have investigated the factors controlling the periodicity of other avian malarias helps little towards the

explanation of these results. Periodicities of 24 hours may be determined by a recurring physiological state of the host, perhaps in relation to its feeding (Boyd, 1933) or to its body-temperature (Stauber, 1939). It is difficult to postulate a recurring physiological condition as determining a 36-hour periodicity, and Stauber (1939) explains such a periodicity as inherent in the parasite itself or as due to its being contained in an abnormal host. It is not easy, however, to reconcile such a view with our observation that intravenous introduction of identical inocula of parasitized blood produced in two fowls infections 24 hours out of step in their periodicity. It would be expected that, if the periodicity were inherent in the parasite itself, these infections, made with parasites at identical stages of the cycle, would have been synchronous when parasites appeared in the peripheral blood. It seems necessary to assume that some deciding factor in the physiology of the host resulted in reversal of the periodicity. The fowls were kept under ordinary day and night conditions during the prepatent period. They were, of course, disturbed during the apparent infection when films were made at 1 a.m. If, however, a certain physiological state occurring in fowls every 36 hours is responsible for the determination of the trophozoite infections, it is difficult to understand why this did not operate in the case of sporozoite-infected fowls also.

The Sexual Forms

The production of gametocytes in malaria in canaries caused by *Plasmodium cathemerium* Hartman or *P. relictum* var. *matutinum* Huff has been investigated by Huff (1927), Shah (1934) and, in greatest detail, by Gambrell (1937). The general conclusions to be drawn from their work are : (1) Gametocytes appear in the peripheral circulation usually simultaneously with the asexual forms. (2) Gametocytes increase and decrease in number with the asexual forms, and both reach a maximum at about the same time. (3) A daily maximum in numbers of gametocytes occurs coincidently with schizogony, but these gametocytes are not fully developed until 6–10 hours later, i.e., if gametocytes are produced, as seems to be generally accepted, from merozoites, a merozoite takes 30–34 hours to develop to a gametocyte and only 24 to schizogony. (4) Long-continued passage by subinoculation tends to decrease the numbers of gametocytes (*P. cathemerium*).

The numbers of gametocytes in all these studies are expressed in relation to the erythrocytes. The erythrocyte count during the apparent infection of *P. praecox* (? = *P. relictum*) in canaries may be reduced, at its lowest level, almost to half the normal count, which is about 5 million per c.mm. (Ben-Harel, 1923).

1. *Appearance of gametocytes at the beginning of the infection with* P. gallinaceum. In the three fowls infected by intravenous inoculation of parasitized blood, which were examined in the greatest detail, gametocytes were found in

thin films of comb-blood very soon after the first parasite was detected. The intervals were actually 33, 40 and 56 hours. The numbers of parasites per 1,000 erythrocytes present in these three cases when gametocytes were first noted were 23, 20 and 37 respectively. In *P. gallinaceum* the proportion of gametocytes to other forms is always low, so that it is probable that gametocytes actually appeared in the peripheral circulation at the same time as the asexual forms, but were not detected earlier owing to their small numbers. We did not, however, feed mosquitoes on these fowls at this stage, and so cannot confirm this supposition by showing the hosts to be infective to a vector.

A similar state of affairs existed in mosquito-infected fowls. After the appearance of the asexual parasites in thin blood-films, gametocytes were noted subsequently as follows : in 1 fowl after 48 hours ; in 1 fowl after 40 hours ; in 1 fowl after 32 hours ; in 5 fowls after 24 hours ; in 1 fowl after 8 hours ; in two further cases gametocytes were found in the first film which showed asexual parasites. The time of appearance of asexual parasites in these cases may be defined as the earliest occasion on which parasites could be found by a 10-minutes' examination of a thin film—an examination which involves the survey of about 20,000 erythrocytes.

Further evidence of the simultaneous appearance of gametocytes and asexual forms was obtained by mosquito-feeding experiments. Study of figs. 2–5 shows that the average oöcyst count of the first batch of mosquitoes fed in each case had reached an appreciable value in all cases 16–24 hours before there were sufficient parasites in the blood to make a reasonably accurate count. When these batches of mosquitoes were fed, only about 15 parasites could be found by a 10-minutes' examination of a thin film, i.e., there was less than one parasite per 1,000 erythrocytes. In one case, three batches of mosquitoes were fed on a fowl at 8-hour intervals, the last batch 16 hours before any parasites were detected. None of these became infected. From these data it is almost certain that gametocytes and asexual forms appear simultaneously in the peripheral circulation, so that fowls are infective to mosquitoes fed upon them from the beginning of the apparent infection.

2. *The proportion of gametocytes to other forms.* Huff (1927) noticed that the percentage of gametocytes among other forms gradually rose as the apparent infection (*P. cathemerium*) progressed. The infection was made by subinoculation. The percentage of gametocytes reached a high value—in one case 80 per cent.—at the end of the primary infection, at a time when the total number of parasites present had fallen to a low level. This occurrence, however, was not repeated in a sugar-induced relapse, when the gametocyte proportion was always below 15 per cent. In our experience, the proportion of gametocytes to other forms in *P. gallinaceum* is always low. Hence it is extremely difficult to obtain reliable values of the gametocyte percentage, especially at the beginning and end of the infection, when the total number of parasites is low. We have therefore, in Table IV, summarized the data relating to gametocyte occurrence in three

subinoculated and two mosquito-infected fowls, these being the sole examples in which the infection was followed in detail and ended in recovery. The total number of gametocytes and of all parasites seen in three films (in one case four), made each day are recorded, and the percentage of gametocytes for each day is derived from these values. It is obvious that all days are not alike in their

TABLE IV

Showing daily percentage of gametocytes of total parasites during five infections followed by means of thin films. Percentage of gametocytes derived from number seen and total number of parasites examined in all three (in one case, four) films in one day. Fowls 20, 24, 25 were subinoculated ; fowls 74, 76 were mosquito-infected

Subinoculated fowls						Mosquito-infected fowls					
Fowl no.	Day after inoculation	Parasites per 1,000 erythrocytes ; 9 a.m.	Total no. of parasites examined	Total no. of gametocytes seen	Percentage of gametocytes	Fowl no.	Day after infection	Parasites per 1,000 erythrocytes ; 9 a.m.	Total no. of parasites examined	Total no. of gametocytes seen	Percentage of gametocytes
20	10	10	216	0	0	74	10	<30	114	2	1·8
	11	42	514	5	0·97		11	92	250	1	0·4
	12	190	800	2	0·25		12	700	500	7	1·4
	13	204	850	4	0·47		13	325	600	5	0·83
	14	238	800	7	0·87		14	43	175	0	0
	15	18	200	7	3·5		15	12	50	0	0
24	6	10	75	0	0	76	11	32	175	2	1·1
	7	30	400	2	0·5		12	172	250	5	2·0
	8	25	600	10	1·7		13	232	350	2	0·57
	9	68	600	14	2·3		14	107	200	1	0·50
	10	36	500	34	5·7		15	63	225	2	0·89
	11	4	120	14	12						
25	6	20	300	4	1·3						
	7	188	600	5	0·83						
	8	640	900	10	1·1						
	9	390	850	8	0·94						
	10	320	600	10	1·7						
	11	48	600	13	2·2						
	12	16	400	13	3·3						

relation to schizogony, but this should not be sufficient to obscure any marked rise in the proportion of gametocytes generally. The subinoculated fowls were about 16 weeks old and were infected at, respectively, the first passage (fowl 20) and the third passage (fowls 24 and 25) from a mosquito-infection. The mosquito-infected fowls (74 and 76) were about 12 weeks old.

Although the number of fowls which yielded data on this point is small, there was in all the subinoculated fowls a slight but constant rise in the gametocyte percentage as the infection progressed to recovery. This rise is comparable to that described by Huff for *P. cathemerium*, but is not so marked. It is noticeable, further, that this constant rise was not present in either of the two fowls infected by mosquitoes, i.e., the gametocyte percentage behaved as in the sugar-induced relapse described by Huff.

3. *The relation of gametocyte production to schizogony.* If gametocytes are produced from merozoites and develop in the peripheral blood, it would be expected that the percentage of gametocytes would show an increase every 36 hours at about the same time as the schizont percentage is rising. This also

TABLE V

Showing variations in percentage of gametocytes among total parasites during the period between times of maximum schizogony. Compiled from data of two subinoculated and six mosquito-infected fowls

Time after schizogony; hours	Subinoculated fowls			Mosquito-infected fowls		
	Total no. of parasites examined	Total no. of gametocytes seen	Percentage of gametocytes	Total no. of parasites examined	Total no. of gametocytes seen	Percentage of gametocytes
1	700	28	4	1,770	18	1·0
5	650	16	2·5	1,825	13	0·71
9	1,000	21	2·1	1,333	7	0·52
13	600	11	1·8	1,815	19	1·0
17	700	10	1·4	1,140	11	0·96
21	675	13	1·9	1,496	16	1·1
25	1,000	18	1·8	1,195	6	0·50
29	650	9	1·4	1,450	13	0·90
33	620	15	2·4	1,601	21	1·4

entails the assumption that they develop from merozoites in about the same time as do schizonts. Such a rise in the percentage of gametocytes, more or less concurrent with the rise in schizont percentage, has been demonstrated by Huff (1927), and can be derived from the results of Gambrell (1937) for *Plasmodium* spp. in canaries. The proportion of gametocytes in the case of these parasites is fairly easily measurable, as it often rises to 30 per cent. In *P. gallinaceum*, on the other hand, the proportion is so low that to demonstrate such an effect in any one given cycle would necessitate the examination of enormous numbers of parasites. We have, therefore, in Table V and fig. 6 summarized data derived from several fowls throughout their infection, arranging the results with relation to time after the previous period of maximum schizogony, which was assumed to fall precisely at midnight or midday.

In the case of fowls infected by subinoculation there is a well-marked rise in the proportion of gametocytes as schizogony approaches (fig. 6). We have shown above that the number of parasites per 1,000 erythrocytes may fall during part of the interschizogony period. If there were selective destruction by the host of asexual rather than sexual forms, this might produce a spurious rise in the gametocyte proportion. As, however, there seems to be no reason to assume this, and as the increase in schizont percentage at the same period is undoubtedly due to the coming to full growth of developing trophozoites, it is reasonable to suppose that the increase of the gametocyte proportion is due to the same cause and not to selective destruction. The maximum, 4 per cent.,

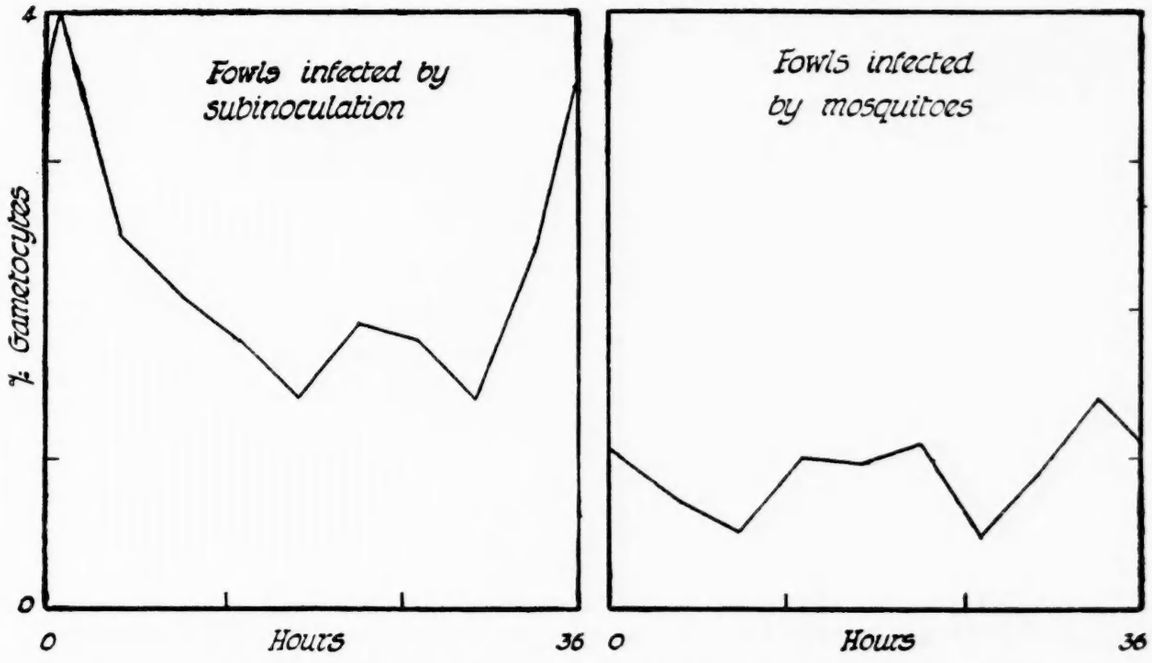


FIG. 6. Graphs showing variations in percentages of gametocytes among total parasites during the period between times of maximum schizogony. Curves constructed from data in Table IV.

is reached about an hour after schizogony, and a rapid fall in the percentage of gametocytes takes place immediately afterwards. This fall is, of course, due to the rapid increase of asexual parasites which takes place at that time. A similar effect is noticeable in mosquito-infected fowls, but in this case the change is not nearly so well marked, a result which is to be correlated with the much lower degree of synchronization which we have found to occur in this type of infection.

In both these curves there is a subsidiary rise which takes place about the middle of the interschizogony period (fig. 6). This occurrence cannot be entirely fortuitous, as it occurs in both curves and both are founded on large numbers of parasites. Such an effect would be produced if the gametocytes took, on an average, longer to develop fully than schizonts, and if a given brood reached full development over a period which, starting before the period of maximum

schizogony, extended for about 24 hours after it. On this hypothesis, the primary rise just before schizogony and the subsidiary interschizogony rise are both caused by the coming to full development of the same brood of gametocytes, but the parts are dissociated by the fall which takes place when the gametocytes are swamped by the rapid increase of asexual forms at the time of schizogony. Our results with *P. gallinaceum*, therefore, agree with those of Gambrell (1937) for *Plasmodium* spp. in canaries.

4. *The gametocyte content of a unit volume of blood.* The changes in the gametocyte content of a unit volume of blood during an infection may be the result of the interaction of several factors: (1) variation in the number of erythrocytes per unit volume; (2) variation in the proportion of parasites to erythrocytes; (3) variation in the proportion of gametocytes to other forms of the parasite.

As the interaction of these factors is highly complex, and especially as the percentage of gametocytes among other forms is extremely difficult of exact determination, we shall not attempt to relate the gametocyte content of the blood to recurring schizogony periods, but will merely point out its general trend during an infection. Study of this point has been carried out only in fowls infected by mosquitoes (fowls 76, 74, 75 and 73; see figs. 2–5). The technique, as noted above, consisted of the counting of gametocytes in 0·0025 c.mm. of blood, so that the values recorded for 1 c.mm. are only approximate but are sufficient to show any wide variations in the gametocyte content. Gametocytes appeared in the counts very early in the course of the infection, and increased irregularly to a maximum, of which the magnitude was very variable but which occurred at a fairly constant time in relation to the total parasite curve—in three cases (fowls 76, 74 and 75; see figs. 2–4) 8–16 hours before the curve reached its maximum level. This is to be explained by the massive blood destruction at the high levels of parasitization, resulting in an actual diminution in the total number of parasites per unit volume of blood. The levels of these maxima of gametocytes per unit volume of blood varied widely (figs. 2–5) and did not necessarily correspond with the highest average oöcyst counts in batches of mosquitoes fed at the time of their occurrence. In the three fowls which recovered (nos. 76, 74 and 75; see figs. 2–4), the gametocyte content then declined irregularly, until sexual forms disappeared from the counts shortly before the estimation of total parasites could be continued. In fowl 73, which subsequently died, the gametocyte content fell and continued at a low level—usually below 2,000 per c.mm.—until death took place (fig. 5).

5. *The infectivity of the blood for mosquitoes.* Attention has already been drawn to the infection of mosquitoes as soon as any parasites are to be seen in films of the peripheral blood. After this time the oöcyst count generally rises to a maximum, which is attained before the total parasite count reaches a maximum. The maximum average oöcyst count varied in the four fowls (nos. 76, 74, 75 and 73) shown in figs. 2–5 from 40 to over 80, and it is noticeable that

the highest maximum average oöcyst count did not occur in the fowl which showed the highest maximum number of parasites per 1,000 erythrocytes. In fact, rather the reverse was the case: in fowl 76 (fig. 2), whose maximum parasite count per 1,000 erythrocytes was only 284, the maximum oöcyst count was 62, while in fowl 75 (fig. 4), which reached a maximum parasite count of 1,010 per 1,000 erythrocytes, the maximum average oöcyst count was only 40. Again, fowl 74 (fig. 3) showed the highest average oöcyst count encountered (83), but the total parasite count only reached 700 per 1,000 erythrocytes at its highest level. This condition has been observed in other fowls besides these quoted here as examples. If a low maximum parasite count is to be interpreted as indicating a high degree of efficiency of the immune mechanism of the host, it seems probable that the gametocyte production of the parasite is stimulated by these, for the parasite, adverse conditions.

The average oöcyst count tends to show a peak in each interschizogony period, a fall taking place before each time of maximum schizogony. This effect is most clearly shown in figs. 2 and 4. Such a result corresponds with the hypothesis stated above, that gametocytes are produced in broods which reach full development in the period of about 24 hours following each time of maximum schizogony. The rises in the average oöcyst-count curve therefore probably correspond with successive broods of gametocytes. If this effect were due merely to the appearance of a brood, resulting in a rise in the average oöcyst count, and if its subsequent removal by the immune mechanism of the host resulted in a corresponding fall, one would expect to find a related rise and fall in the gametocyte content per unit volume. Although the gametocyte-count technique admittedly gives only an approximation of the gametocyte content, there is a pronounced lack of relation between the latter and the average oöcyst count in all the fowls studied. Only in one case (fowl 74, fig. 3) does the maximum gametocyte content occur at the same time as the highest average oöcyst count, and in this case the gametocyte count was extremely high. This is considered to indicate that some other factor operates besides the simple removal of gametocytes from the circulation by the host—perhaps that the gametocytes are only capable of infecting mosquitoes for a short time after reaching full development, and that many of the gametocytes in the peripheral blood are non-viable for a time before they are removed by the phagocytic mechanisms of the host. Such an occurrence would explain also why the highest average oöcyst count usually precedes the maximum gametocyte content per unit volume.

In the two fowls (nos. 76 and 74) whose infection was followed right through to recovery, the average oöcyst count fell very suddenly at the end of an infection to a low level (figs. 2, 3). Nevertheless, both these fowls continued to infect mosquitoes after the parasite counts had to be discontinued, although the average oöcyst count was low (figs. 2, 3).

In fowl 73, whose infection terminated fatally (fig. 5), the average oöcyst

count, after passing its maximum, pursued a course at a medium level until death took place.

SUMMARY

1. The biology of *Plasmodium gallinaceum* Brumpt, 1935, is studied in young domestic fowls, infections being produced both by subinoculation and by mosquito-infection with *Aëdes aegypti* (L.). Counts are recorded which show variations during the apparent infection in : (*a*) the total number of parasites per 1,000 erythrocytes ; (*b*) the percentages of trophozoites, schizonts and gametocytes among total parasites ; and (*c*) the number of gametocytes per c.mm. of blood. The average number of oöcysts present on the mid-gut wall in batches of *Aëdes aegypti* 10 days after feeding on a fowl is taken as an index of the number of gametocytes, capable of further development in the mosquito, which were present in the peripheral blood at the time of feeding.

2. The length of the prepatent period following infection by subinoculation is very variable. The mean prepatent period in fowls 5–6 weeks old infected by the inoculation of heavily parasitized blood is $6{\cdot}41 \pm {\cdot}34$ days when the intramuscular route is used (29 instances), and $2{\cdot}75 \pm {\cdot}19$ days when injection is made intravenously (16 instances). This difference is significant. The prepatent period in fowls infected by mosquitoes is very uniform : in 16 fowls 8–16 weeks old, the mean prepatent period is $9{\cdot}0 \pm {\cdot}15$ days.

3. No obvious alteration is noted in the course of the apparent infection, whether it is produced by subinoculation or by mosquitoes. The courses of typical infections ending in death and recovery are described.

4. The conclusions of Giovannola (1938) that the periodicity of *Plasmodium gallinaceum* is one of 36 hours, with periods of maximum schizogony falling at midnight and midday alternately, are confirmed.

5. The degree of synchronization of reproduction of *P. gallinaceum*, as judged by the maximum percentages of schizonts occurring, is greater in subinoculated fowls than in those infected by mosquitoes.

6. The periods of maximum schizogony occur at definite times after infection by mosquitoes. In all of 12 fowls infected in this manner a period of maximum schizogony occurred about midday on the 13th day after inoculation. The preceding or succeeding periods were at times separated from midday on the 13th day by multiples of 36 hours. In subinoculated fowls no such regularity is present : in three fowls in which the infections were closely followed, each fowl fulfilled one of the three different theoretical possibilities, i.e., on the 10th day after inoculation, when the infection was apparent in all three fowls, schizogony took place in one instance at midday and in the other two at the preceding and succeeding midnights respectively.

7. Gametocytes appear in the peripheral circulation at the beginning of the apparent infection with *P. gallinaceum* as soon as do asexual forms.

8. The percentage of gametocytes among total parasites in *P. gallinaceum* is always low—usually about 1–2 per cent. Towards the end of infections progressing to recovery, the percentage of gametocytes tends to rise when the infections have been produced by intravenous inoculation, but not in those following mosquito-infection.

9. The percentage of gametocytes among total parasites rises at about the same time as the percentage of schizonts, and a subsidiary rise takes place in the middle of the interschizogony period. This is considered to indicate that gametocytes are probably produced in broods which reach maturity during the period of about 24 hours succeeding schizogony.

10. Fowls are infective to *Aëdes aegypti* (L.) from the beginning of the apparent infection with *P. gallinaceum*. The highest average oöcyst count tends to precede the highest gametocyte count per unit volume. The curve of the average oöcyst counts tends to show a peak in each interschizogony period, presumably corresponding with the coming to full development of a brood of gametocytes.

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THE EFFECT OF PLASMOQUINE AND OF PRAEQUINE ON THE SUBSEQUENT DEVELOPMENT OF THE GAMETOCYTES OF *PLASMODIUM GALLINACEUM* BRUMPT, 1935, IN *AËDES AEGYPTI* (L.)

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INTRODUCTION

The present investigation was originally undertaken to compare the action of the German drug plasmoquine with that of praequine, the equivalent French drug, on the gametocytes of *Plasmodium gallinaceum* Brumpt, 1935, in the domestic fowl. These two drugs are considered to be of identical chemical constitutions, being both 6-methoxy quinoline with a long open chain system in the 8N position (Field, 1939). As a basis for a study of this kind, it was considered necessary in the first place to investigate the biology of the parasite, particularly with respect to gametocyte production, so that the action of gametocidal drugs could be more accurately assessed. The latter work has already been reported (Lumsden and Bertram, 1940). Reference may be made to that paper for the general technique adopted ; only special methods in connection with drug-administration will be mentioned below.

The efficacy of a gametocidal drug may conceivably be assessed in several ways. A dose of a drug may produce reduction in numbers or morphological changes of the gametocytes in the peripheral blood. The effects of the drug on the morphology of *Plasmodium* spp. in canaries have been described by Roehl (1926), Wampler (1930), Manwell (1930, 1932), and Manwell and Haring (1938). These authors describe vacuolation and arrest of development of the asexual parasites, but make little reference to effects on the gametocytes. Wampler states that, after large doses of plasmoquine (about 10 mgm. per kilo. orally), the gametocytes of *P. cathemerium* were, though few, apparently normal in films which were made 24 hours after dosage and in which all the asexual parasites were in a state of degeneration. Manwell disagrees with this statement, and maintains that gametocytes are at least as susceptible as asexual forms. Brumpt *et al.* (1937), with reference to *Plasmodium gallinaceum*, state that after

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1 mgm. per kilo. of plasmoquine administered intramuscularly the gametocytes are the first forms to disappear. Kohlschütter (1938) describes vacuolation and clumping of the pigment in the asexual forms of the same parasite four hours after an oral dose of plasmoquine of about 7 mgm. per kilo. He does not record any changes in the gametocytes. With regard to human malaria, plasmoquine causes the disappearance of the gametocytes from the peripheral blood (Field, 1939), but no clear morphological criteria are available to indicate non-viability on the part of the gametocytes. Barber, Komp and Newman (1929), after careful examination of films made after a single dose of plasmoquine (0·859 mgm. per kilo. orally), came to the conclusion that the only measurable effect was a slightly higher percentage of gametocytes which stained more faintly than those in the control. Morphological changes thus were useless for the assessment of the efficacy of a drug, particularly as it was desired to determine whether small doses would render the sexual forms non-viable. Moshkovsky and Burova (1934) record that the male gametocytes of *Haemoproteus* sp. in linnets were prevented from exflagellating in 24 hours by a parenteral dose of 5 mgm. plasmoquine per kilo. Manson-Bahr (1928) states that in human malaria a dose of 0·03 gm. plasmoquine (with quinine sulphate as plasmoquine-compound, probably about 0·5 mgm. plasmoquine per kilo.) inhibited exflagellation of crescents, and, after 0·08 gm. had been administered, degenerative changes appeared in them. Barber, Komp and Newman (1929), however, have shown with *P. falciparum* that a much smaller dose—0·185 mgm. per kilo. body weight —while insufficient to prevent exflagellation, did prevent oöcyst development in *Anopheles albimanus* fed on the patient about 24 hours afterwards. A few workers besides Barber *et al.* (1929) have tested the infectivity of the gametocytes of human malaria by mosquito-feeding experiments after the administration of plasmoquine. The smallest single dose which Whitmore (1929) was able to demonstrate as preventing the gametocytes of *P. falciparum* from developing in *Anopheles albimanus* was 0·326 mgm. per kilo. orally. The mosquitoes were fed on the patient from 11 hours to six days after the dose. Quinine treatment was also carried out during observation. Amies (1930), working with *P. falciparum* in Malaya, experienced difficulty in obtaining cases which would infect mosquitoes regularly before plasmoquine treatment began. Nevertheless, two of his cases infected mosquitoes for three consecutive days before treatment, which consisted of doses twice daily of, in one case, 0·41 mgm. per kilo., and, in the other, of 0·38 mgm. per kilo. orally. Mosquitoes (*Anopheles philippinensis*) were applied daily to these patients after treatment began, the first batch two hours after the second dose. No infected mosquitoes were obtained for two days afterwards in the first case, and for three days in the second. Observations were then discontinued, the number of crescents having fallen to a very low level. Sur, Sarkar and Banerji (1932), using *P. falciparum*, *P. vivax* and *P. malariae*, showed that cases previously infective to mosquitoes became non-infective after three daily doses of about 0·3 mgm. per kilo. orally. Several Bengal

species of *Anopheles* were employed. In many of these cases, however, including some of malignant tertian, the simple plasmoquine treatment abolished both trophozoites and gametocytes from the blood-films. The administration of repeated doses by these workers has obscured decision as to the duration of action of a single dose, but there is evidence from some of their work that viable gametocytes do not reappear for several days after a single effective dose—a result which is to be expected if gametocytes are produced in broods, as has been shown to be the case in some avian malarias. It seems probable that the action of plasmoquine on the gametocytes must take place, in man at least, in the eight hours succeeding administration, as Green (1929) has shown that the drug cannot be detected in the urine after that period. The only workers who have performed similar experiments with avian malaria seem to be Kritschewski and Pines (1934), who, working with *Plasmodium praecox* (? =*P. relictum*) in *Spinus spinus* (the siskin), found that 20 mgm. per kilo. was necessary to prevent the development of oöcysts in *C. pipiens* fed on the birds 12–24 hours after treatment ; 5 mgm. per kilo. was ineffective. This dose is very much higher than those shown to be effective in human malaria.

It seemed, therefore, that the most delicate criterion for the estimation of the action of a drug was the development of the parasite in the mosquito. As we desired to examine the action of a drug as quantitatively as possible, we selected oöcyst counts as the most suitable for our purpose, as doubt has been thrown by Roy (1938) on the validity of counts of sporozoites in the glands ; and in any case the number of sporozoites in a gland are likely to vary from day to day. It must be admitted that the presence of sporozoites in the salivary glands is the ultimate criterion of the viability of the gametocytes taken in by the mosquito, but this does not detract from the quantitative value of an oöcyst count. The general value of oöcyst counts has been discussed in a previous paper (Lumsden and Bertram, 1940).

We have attempted, in the following experiments, to determine in the case of *Plasmodium gallinaceum* how soon a fowl may be rendered non-infective to mosquitoes feeding upon it, and whether viable gametocytes again appear in the circulation after dosage. The study is far from complete, but, the work having to be terminated, it is considered worth while to place on record such results as have been obtained, and to indicate the technique which seems to us, after experience of the course of the infection, most suitable to adopt in further studies directed to these ends.

METHODS OF DOSAGE

Both plasmoquine (Bayer) and praequine (May and Baker) were supplied in 1 per cent. solution in ampoules. The dose was administered orally in the following manner :

A no. 12 rubber catheter was attached to a 20-c.cm. syringe filled with freshly boiled distilled water, and the catheter was filled with water from the

syringe. The catheter was then passed into the crop of the fowl. The required dose was injected from a 1-c.cm. glass syringe by means of a needle passed through the catheter-wall, and was washed into the crop by about 10 c.cm. of water from the syringe. When small doses were to be given, the original solution was diluted appropriately with freshly boiled distilled water. Dilution and injection were carried out in all cases as quickly as possible, as the drug oxidizes rapidly in solution exposed to the air (some other authors have taken little precaution to avoid oxidation). The birds were kept under close observation for some time after treatment, to make sure that no regurgitation took place.

Dosage. The maximum tolerated dose *per os* in canaries is 33 mgm. per kilo. (Roehl, 1926) and in fowls about 29 mgm. per kilo. (Kohlschütter, 1938). In man much smaller doses than these produce toxic signs. Kohlschütter (1938) records a dose of about 14 mgm. per kilo. as effective therapeutically on the general infection (*P. gallinaceum*). This dose was taken as a starting point, and the action of one or both drugs was investigated for about the same dose and for doses of about 1/5, 1/10 and 1/100 of this magnitude. The effects of these doses both on the general infection and on the gametocytes will be indicated as far as possible. Control fowls were used in each experiment, but data relating to them are omitted except when they are significant.

RESULTS

I. Dose : 15 mgm. per kilo.

The two fowls used in this experiment were 8 weeks old and were infected by mosquitoes. One was used as a control and was untreated ; the other received plasmoquine orally.

Parasites appeared in films of the control fowl on the 11th day after infection, and their number increased in the usual manner (see Lumsden and Bertram, 1940) to a maximum of 1,060 parasites per 1,000 erythrocytes on the 14th day. The total parasite count then decreased for 24 hours, but after that time (on the 15th day) it again began to rise. The total parasite count curve continued thereafter to rise slowly and irregularly until death took place at the 22nd day, when about 1,200 parasites per 1,000 erythrocytes were present. During this period of slow rise from the 15th to the 22nd day, the synchronization of the parasite was disturbed and the percentage of schizonts remained irregularly at about 10 per cent. Gametocytes could be found in films throughout the infection, but they were less numerous during the latter part of the infection than before the maximum total parasite count was passed. Batches of mosquitoes were fed on the fowl at about 24-hour intervals throughout the infection. Except in one instance, which will be noted below, oöcysts developed in the mosquitoes of all these batches. The average oöcyst counts also were lower in the latter part of the infection than before the 14th day.

Parasites appeared in the experimental fowl on the 11th day after infection

and increased, in a closely similar manner to the control, to a maximum, on the 14th day, of 720 parasites per 1,000 erythrocytes. The parasite count then began to fall, and 18 hours later, at 7 p.m. on the 14th day, when the drug was administered, had decreased to about 420 parasites per 1,000 erythrocytes. This fall was very similar to that described in the control fowl, but later the parasite count continued to fall ; its decrease was not, as in the control, arrested. Further, it fell so rapidly that on the 16th day, 38 hours after dosage, only about 10 parasites per 1,000 erythrocytes were present. This fall was much more rapid than that which takes place in recovering untreated fowls (Lumsden and Bertram, 1940), and is almost certainly to be attributed to the action of the drug. Scanty parasites were, however, to be found in the blood until at least the 19th day. Between 14 hours and two days after dosage the parasites stained palely and were much vacuolated, and the pigment granules were arranged at their periphery.

Gametocytes were present in the peripheral blood of the experimental fowl before drug-administration in about the same numbers as in the control. Some apparently normal gametocytes could be found six hours afterwards, but no others were seen subsequently, though prolonged searches were made up to the 19th day.

Daily batches of mosquitoes were fed as in the case of the control fowl, and the experimental fowl before treatment was constantly infective. After treatment, batches of mosquitoes were fed on both fowls, the first batch 30 hours afterwards. In this case, however, the mosquitoes could be induced to feed only at the higher temperature and humidity of the insectarium (see Bertram and Gordon, 1939), and those which fed on both fowls showed no oöcysts when subsequently dissected. Perhaps these special conditions resulted in an arrest of oökinete development, but in a subsequent case in which normal conditions were observed 46 hours after dosage no infection followed after feeding on the treated fowl, while the control-fed *Aëdes* showed infection. No further infection was obtained in the case of the treated fowl, though mosquitoes were fed daily until the 20th day.

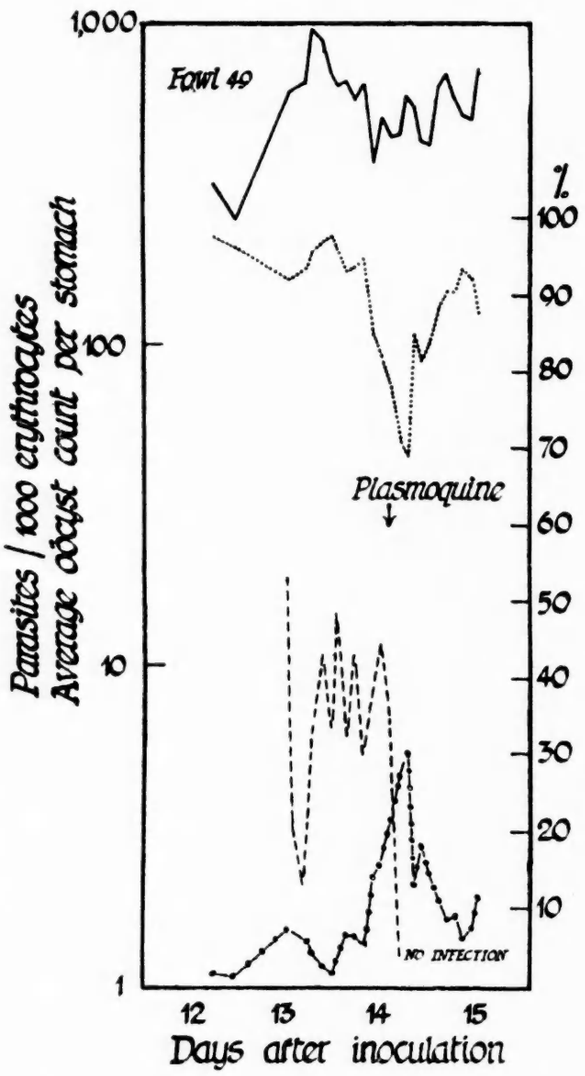
Although the fowl was prevented from infecting mosquitoes by this dose, the amount of drug was sufficient to reduce the total parasites to a very low level, so that the effect is not necessarily a selective one on the sexual forms. Nevertheless, in recovering untreated fowls some mosquitoes are usually infected when the total parasite count has reached a very low level (Lumsden and Bertram, 1940).

II. Dose: 2·8 mgm. per kilo.

The experimental fowl (no. 49) was about 12 weeks old and was infected by mosquitoes. Observations were made about every two hours over a 48-hour period towards the end of an infection which, on the 16th day, ended in death. The observations are summarized in fig. 1. Plasmoquine alone was used,

and the dose was administered 25½ hours after the preceding period of maximum schizogony at about midday on day 13. The dose exerted no obvious effect on the general course of the infection.

The gametocyte counts have been omitted from the graph to avoid confusion; there were, however, 1,000–3,000 present per c.mm. in all counts on day 13, 600–2,400 in all counts on day 14, and less than 1,000 in all counts



FOWL 49: AGE AT INOCULATION (BY MOSQUITO) ABOUT 12 WEEKS; PLASMOQUINE DOSE 2·8 MGM. PER KILO.

FIG. 1. Graphs showing total parasite count and average oöcyst count (logarithmic ordinates) and percentages of trophozoites and schizonts (arithmetic ordinates) in fowl 49 during infection with *P. gallinaceum*.

——— denotes parasites per 1,000 erythrocytes.
– – – – „ average oöcyst count per stomach.
..... „ percentage of trophozoites.
o–o–o–o „ percentage of schizonts.

on day 15. Two counts on the latter day at 5 a.m. and 7 a.m. showed no gametocytes, but they reappeared in subsequent counts.

The behaviour of the fowl as regards the infection of mosquitoes is also shown in fig. 1. The normal fall in the average oöcyst count which takes place about the time of schizogony is seen to occur about midday on day 13. This is followed by the normal rise in the average oöcyst count in the interschizogony period. After the administration of the drug, a brisk fall took place in the count, and the batch which was fed four hours later showed no infection. No oöcysts were found in any subsequent batches, though these were applied to the fowl up to 22 hours after the dose.

A fall in the average oöcyst count is normally to be expected at this time (Lumsden and Bertram, 1940), but, in our experience, it never falls to zero. Further, the subsequent rise did not take place, so that it is certain that this dose of the drug was sufficient to render the gametocytes in the fowl non-infective for mosquitoes.

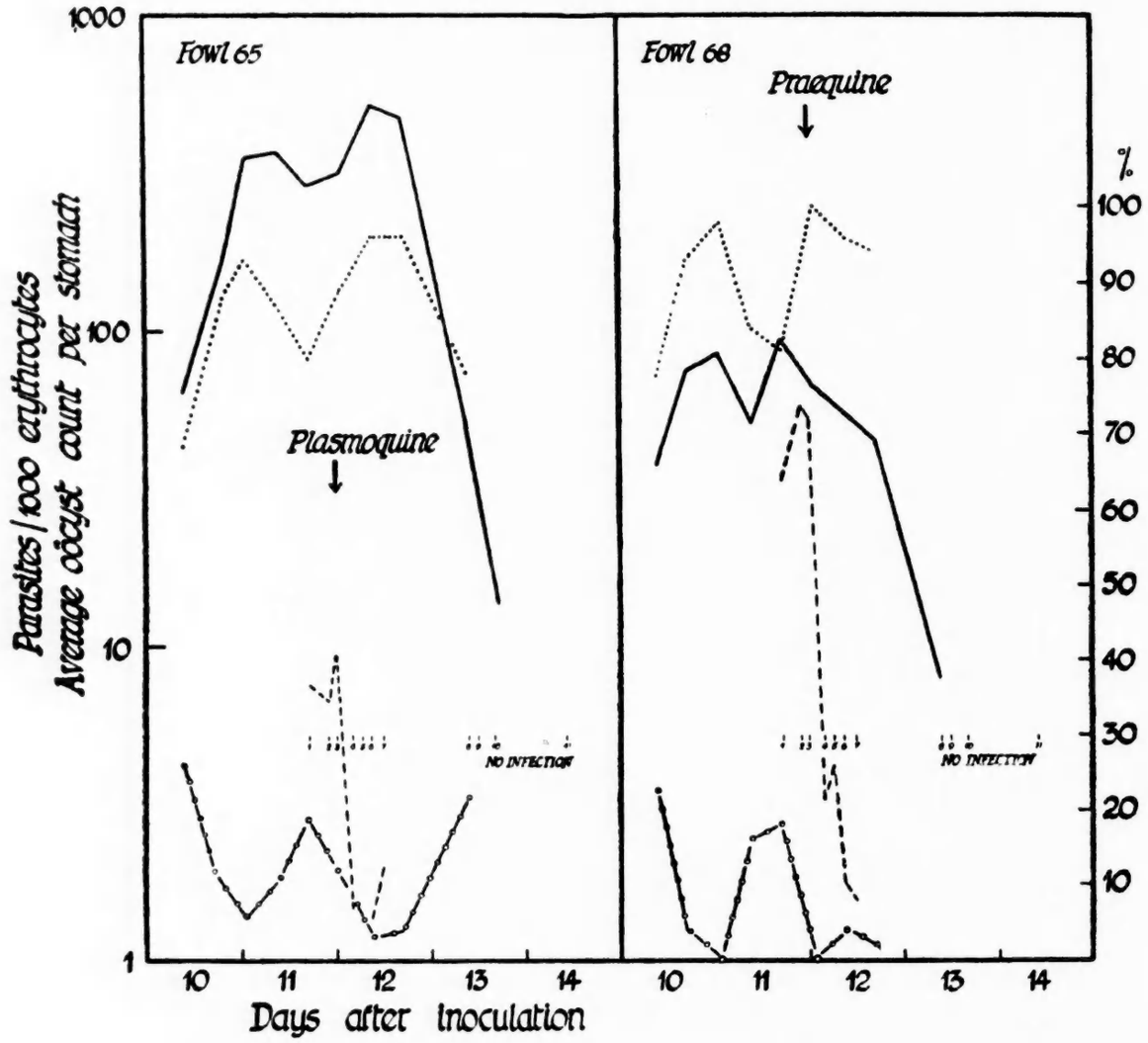
As the fowl died shortly after this, we were unable to determine if the subsequent brood of gametocytes was viable.

III. Dose: 1·4 mgm. per kilo.

The two experimental fowls (nos. 65 and 68) used in this instance were about 12 weeks old and were infected by mosquitoes. Both plasmoquine (fowl 65) and praequine (fowl 68) were used. The course of the infection was followed by means of total parasite counts and differential counts. No gametocyte counts were made. The data are shown in fig. 2, which also indicates the times at which batches of mosquitoes were applied (numbers 1–11). The average oöcyst counts found in these batches are also plotted. The batches of mosquitoes were fed at short intervals for several hours preceding and following the administration of the drug. Subsequent batches were fed about 36 hours and 60 hours later.

The drugs were administered about midnight on day 11–12, i.e., at a time of maximum schizogony. The doses exerted no obvious effect on the general course of the infection. The average oöcyst count fell sharply in both cases. This is especially noticeable as the fall takes place at a time following schizogony, when a rise would rather be expected (Lumsden and Bertram, 1940). However, all the gametocytes were not sterilized in either fowl after 12 hours. Subsequent batches (8–11 ; see fig. 2) did not show any infection, but by that time the total infection had decreased to a level at which even in untreated fowls the average oöcyst count is low. Nevertheless, it is significant that no infection was obtained in these cases ; a comparison may be made between fig. 2 and figs. 2 and 3 in the previous paper (Lumsden and Bertram, 1940).

No significant difference is to be detected between the effect of plasmoquine and that of praequine.



FOWLS 65 AND 68: AGE AT INOCULATION (BY MOSQUITO) ABOUT 12 WEEKS; DOSE OF PLASMOQUINE ADMINISTERED TO FOWL 65 AND OF PRAEQUINE ADMINISTERED TO FOWL 68, 1·4 MGM. PER KILO.

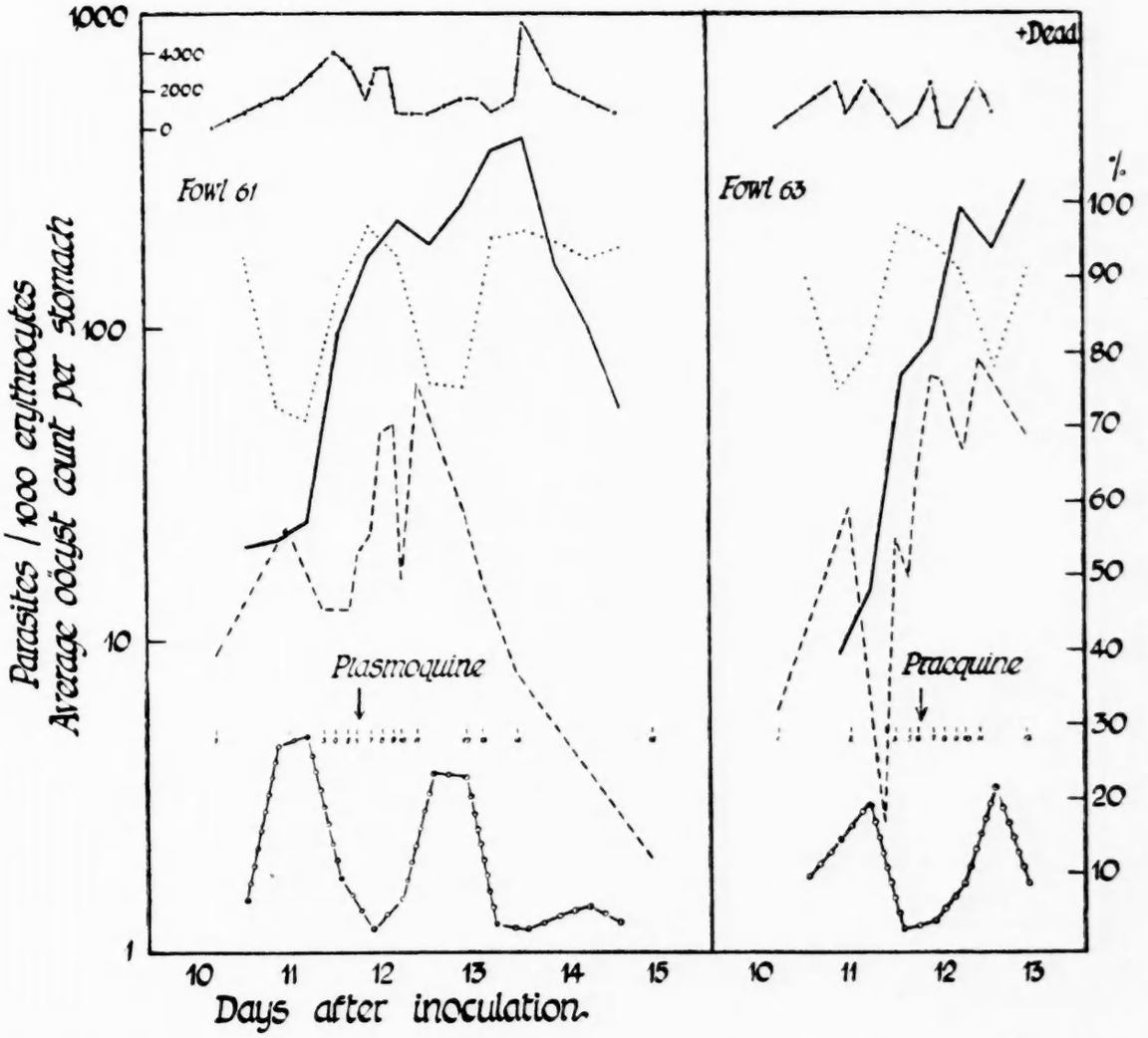
FIG. 2. Graphs showing total parasite counts and average oöcyst counts (logarithmic ordinates) and percentages of trophozoites and schizonts (arithmetic ordinates) in fowls 65 and 68 during infection with *P. gallinaceum*. The times at which batches of mosquitoes were applied are indicated by numbers 1–11.

——— denotes parasites per 1,000 erythrocytes.
– – – – „ average oöcyst count per stomach.
..... „ percentage of trophozoites.
o–o–o–o „ percentage of schizonts.

IV. DOSE: 0·14 MGM. PER KILO.

The two experimental fowls (nos. 61 and 63) used in this instance were about 12 weeks old and were infected by mosquitoes. Both plasmoquine (fowl 61) and praequine (fowl 63) were used. The same technique was adopted as in the last experiment. The data are shown in fig. 3.

The doses exerted no obvious effect on the general course of the infection, and are also seen to have been inadequate to prevent massive oöcyst development in mosquitoes fed on the fowls at intervals for 16 hours afterwards. Mosquitoes continued to become infected from fowl 61 (plasmoquine) for more than three days, and probably the same would have been the case with fowl 63 (praequine),



FOWLS 61 AND 63: AGE AT INOCULATION (BY MOSQUITO) ABOUT 12 WEEKS; DOSE OF PLASMOQUINE ADMINISTERED TO FOWL 61 AND OF PRAEQUINE ADMINISTERED TO FOWL 63, 0·14 MGM. PER KILO.

FIG. 3. Graphs showing total parasite counts and average oöcyst counts (logarithmic ordinates) and percentages of trophozoites and schizonts (arithmetic ordinates) in fowls 61 and 63 during infection with *P. gallinaceum*. The times at which batches of mosquitoes were applied are indicated by numbers 1–15.

——— denotes parasites per 1,000 erythrocytes.
– – – – „ average oöcyst count per stomach.
····· „ percentage of trophozoites.
o–o–o–o „ percentage of schizonts.
·–·–·–· „ gametocytes per c.mm.

but it died on the 13th day. These doses had no apparent effect on the gametocyte counts (fig. 3); neither were any morphological changes seen in the gametocytes.

V. Experiments to determine if plasmoquine in the amounts taken up by a mosquito from a treated fowl exerts any action on the development of the parasite in that host

The problem was approached from two directions:

1. A number of mosquitoes were fed on a fowl during its apparent infection. Four days later the mosquitoes were divided into three batches and were fed as follows: (*a*) on an uninfected untreated fowl; (*b*) on an uninfected fowl about eight hours after it had received a dose of plasmoquine of 14 mgm. per kilo. orally; (*c*) on an uninfected fowl about eight hours after it had received a dose of plasmoquine of 2·8 mgm. per kilo. orally.

All these batches of mosquitoes developed oöcysts, so that there is insufficient drug in the amount of blood taken up by *Aëdes aegypti* from a treated fowl eight hours after dosage to arrest the further development of 4-day-old oöcysts.

2. Three batches of mosquitoes were fed as (*a*), (*b*) and (*c*) in the last experiment. Four days later these batches were all fed on an infective fowl.

All these batches developed oöcysts, so that insufficient drug persists in *Aëdes aegypti* for four days after feeding on a treated fowl to prevent oökinete development when fed on an infective fowl.

DISCUSSION

The conclusions as to the efficacy of plasmoquine and praequine in the prevention of mosquito infection are set out above. The question of whether or not viable gametocytes reappear in the circulation at a later date has not been answered, as the infection usually died out naturally before such observations could be carried out. As we have shown, from the study of untreated infections, that gametocytes appear at the beginning of the apparent infection and that the average oöcyst count early reaches an appreciable value (Lumsden and Bertram, 1940), it would be more satisfactory for the assessment of the efficacy of a drug if it were administered much earlier in the infection—say, in the type of infection which we have described in our previous paper, at midday on the 10th day. If the infection followed its normal course and the average oöcyst count fell after administration and no mosquitoes were infected before midnight on day 11–12, it could fairly be said that the dose was sufficient to sterilize all that brood of gametocytes. Further batches of mosquitoes could be applied at about midday on day 12, at about midnight on day 13–14, and at about midday on day 15, to determine if any of the successive broods of gametocytes were viable. In most cases, then, the viability of four successive broods of gametocytes could be determined.

SUMMARY

1. The effects of the drugs plasmoquine and praequine on the subsequent development of the gametocytes of *Plasmodium gallinaceum* Brumpt, 1935, in *Aëdes aegypti* (L.) have been investigated.

2. Plasmoquine—15 mgm. per kilo. orally—produces rapid diminution of the general infection ; no mosquitoes are infected 46 hours to six days after the dose. Though the level of parasitization is low, parasites are present in the blood for at least five days after the dose.

3. Plasmoquine—2·6 mgm. per kilo. orally—produces no obvious effect on the general infection, but no mosquitoes are infected between 4 and 22 hours after the dose.

4. Plasmoquine or praequine—1·4 mgm. per kilo. orally—produce no obvious effect on the general infection, and, though a fall in the average oöcyst count occurs, complete sterilization is not produced in 12 hours. No difference can be detected between the actions of the two drugs.

5. Plasmoquine or praequine—0·14 mgm. per kilo. orally—produce no obvious effect on the general infection ; neither are they able to prevent massive infection of mosquitoes up to 16 hours after dosage.

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STUDIES IN CHEMOTHERAPY

XXV.—A SECOND CASE OF INDIAN KALA-AZAR TREATED WITH 4:4′-DIAMIDINO STILBENE

BY

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AND

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In a previous paper (1939) we have recorded the successful treatment of a case of Indian kala-azar with an aromatic diamidine compound, viz., 4 : 4′-diamidino stilbene; the present note gives details of the result of treating a second case of Indian kala-azar with this compound.

The patient was a Hindoo seaman, aged 26, from Calcutta, who a week previously had been admitted to a local hospital with fever and debility of unknown cause, but suspected to be of malarial origin. He was unable to speak English, and it was therefore impracticable to obtain a history other than that he was sailing regularly to India and had had ' fever ' for about three months, with some pain over the splenic area.

On admission to the Tropical Ward of the Liverpool School of Tropical Medicine on June 4th, 1940, the temperature was of a remittent type ranging several degrees daily to 102° or 103° F.; the patient looked ill and anaemic, but was not unduly emaciated, although his weight was only 43·5 kilo. There were some patches of dusky pigmentation over the forehead and malar bones. The spleen was enlarged and firm, and extended one inch below the umbilicus; the liver was not palpable.

The blood picture was as follows:

Red cells ..	1,700,000		
White cells..	2,500	Neutrophils ..	26 per cent.
		Eosinophils ..	0 ,,
		Basophils.. ..	0 ,,
		Small lymphocytes	38 ,,
		Large ,,	20 ,,
		Monocytes ..	16 ,,

Haemoglobin 26 per cent. and colour-index 0·76.

The formol-gel test was strongly positive. *Leishmania donovani* were easily found in smears of spleen pulp, but in sternal marrow puncture smears no parasites could be found on long search.

On June 10th, 1940, a course of intravenous injections of 4 : 4′-diamidino stilbene was begun. The dosage was about 1 mgm. per kilo. daily for 8 days, and the total amount of drug injected was 400 mgm. The only apparent immediate result of the treatment was an increase in the daily excursions of the

temperature, which fluctuated from normal to 104° F. for the first three or four days; thereafter the temperature steadily fell, until on the seventh day of treatment it became normal and remained so until the patient was discharged. With the fall in temperature the patient's general physical condition showed improvement, although throughout his stay in hospital his weight did not increase. On June 19th, the formol-gel test was positive, although not so markedly as on admission. On June 24th, the spleen had shrunk ½–1 inch all round its palpable border; and on June 27th it had retracted to a point midway between the umbilicus and the costal margin. A few days later it was only two fingers below the costal margin, but no further shrinkage occurred up to the time of the patient's discharge, a month later.

On July 3rd, 1940, the blood picture was as follows:

Red cells ..	3,200,000		
White cells ..	5,500	Neutrophils ..	63 per cent.
		Eosinophils ..	0 ,,
		Basophils	1 ,,
		Small lymphocytes	20 ,,
		Large ,,	10 ,,
		Monocytes ..	6 ,,

Haemoglobin 50 per cent. and colour-index 0·8.

The formol-gel test at this time was doubtfully positive.

On July 12th, sternal marrow and spleen puncture material smears were examined and no Leishman-Donovan bodies were found. Culture of these tissues was negative. The patient now had become very lachrymose on account of his isolation from his fellow-countrymen, and was not taking his food. He left hospital on July 22nd in good general physical condition and apparently perfectly well, although his weight had not materially increased.

SUMMARY

This note records the apparent cure of a second case of Indian kala-azar by an aromatic diamidine, viz., 4 : 4′-diamidino stilbene.

REFERENCE

Adams, A. R. D., and Yorke, W. (1939). Studies in chemotherapy. XXIII: A case of Indian kala-azar treated with 4 : 4′-diamidino stilbene. *Ann. Trop. Med. & Parasitol.*, **33**, 323.

AN EPIDEMIC OF KALA-AZAR IN THE UPPER NILE PROVINCE OF THE ANGLO-EGYPTIAN SUDAN

BY

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(*Received for publication August 14th*, 1940)

INTRODUCTION

From the distribution of leishmaniasis in the Sudan it would appear that the main endemic area was along the Abyssinian border and that spread occurred along the course of the rivers. But in recent years sporadic cases have been found over the whole country, and it is probable that there are few areas where this sporadic form does not occur.

As far as the Upper Nile Province is concerned, cases have been found throughout the area north of the River Sobat-Lake No line, and although no cases have yet been recorded further south there is no valid reason for supposing that the disease does not occur there, as it is found in adjacent areas in other provinces.

The epidemic dealt with in this paper occurred amongst a small section of the Dinka tribe inhabiting an area east of the White Nile some hundred miles north of Malakal.

TERRAIN AND POPULATION

The total population of the area is about 8,000, and the country which these people inhabit comprises a narrow strip of wooded and, for five months of the year (July-November), swampy riverain land, with a hinterland which is in the main flat grassland plain, crossed by two rivers and several streams, all of which are dry for five to six months of the year (January-June). To the east is a large area of low-lying grassland—the Subat—which is an enormous swamp during and for some time after the rainy season, but which affords grazing for a large number of cattle from February to June inclusive.

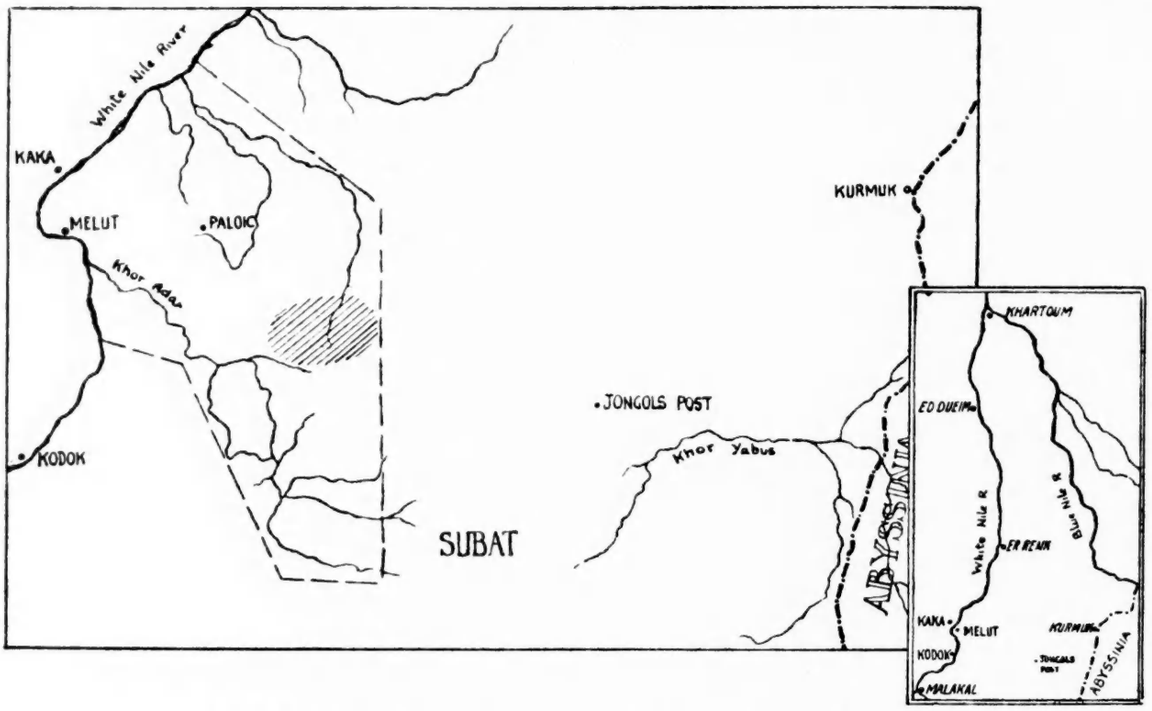
Although the Dinka are essentially cattle-owning people, this section of them is partly agricultural in habit. From June until the end of December, they dwell in villages scattered over the hinterland, and are mainly engaged in cultivating millet. At the end of December, when the main water-supplies begin to dry up, the cattle are moved down to camps on the river, and soon afterwards practically the whole population follows, remaining in the camps until the beginning of June. From a group of villages in the east, however, the cattle are taken down to the Subat for their dry-weather grazing.

It has been suggested that the site of infection is more likely to be the

riverain land than the hinterland, but this is hardly tenable, as the large majority of cases occurring in this epidemic came from the eastern group of villages, whose people do not go down to the river camps.

HISTORY

Archibald (1923) reported an outbreak among troops stationed at Jongols Post ' in the Melut district.' Jongols is in an area inhabited by a tribe called the Maaban, situated some distance to the east of the area under consideration



Map showing part of the southern Sudan. The district in which the epidemic occurred is indicated by the interrupted line. The shaded area represents the eastern group of villages in which most of the cases occurred. The inset shows the position of the district in the Sudan.

in the present paper, and separated from it by a dry uninhabited plain some 30 miles wide. In the past there was little contact between the Dinka and the Maaban, but of recent years this has increased, and it is considered probable that the original infection in this epidemic came from the Maaban.

The first cases of kala-azar were discovered in this area in 1932, but only a few cases were recorded each year until 1936, when reports were made to the medical authorities that a large number of people had died as a result of the disease. It was not until the end of 1937 that cases came into hospital for treatment in any numbers, but in the 18 months following 73 cases came under notice. During 1939 the epidemic abated, and it would now (February, 1940) appear to have come to an end. It is estimated that in the space of three years at least 300 cases must have occurred, and the death-rate was probably 80 per cent. Both these figures are considered to be low estimates.

PREDISPOSING CAUSES

The general standard of living of these people is low. It is probable that their diet is deficient in several respects, and practically every year for a period of about two months before their first harvest (November) they are in a state of semi-starvation. In particular, it is considered probable that their diet is deficient in adequate supplies of vitamin C, and that many of the people are on the threshold of avitaminosis. The area is highly malarious, with an infection-rate of probably 100 per cent.

DISTRIBUTION

Of the 73 cases recorded, 46 (63 per cent.) came from a group of villages in the eastern part of the area, comprising at most one-sixth of the total population, the remaining cases being scattered over the rest of the area; and it is considered that this is more or less representative of the distribution of the disease as a whole.

In the eastern area, about one-third gave a history of other people in the same house being affected, but in the rest of the area the proportion was much less.

ANALYSIS OF CASES

Of the 73 cases recorded, 19 either died before treatment could be commenced or left hospital having had little or no treatment. Results of the remaining 54 cases can be seen in the following table.

Admitted to hospital	Died in hospital	Discharged as presumably cured	Left hospital without completing treatment	Total survivors in Jan., 1940
54	25	22 of which 11 died later	7 of which 6 died later	12

The age- and sex-distribution of cases correspond to the figures of hospital admission in general, and the impression gained on the spot was that the disease attacked young and old, male and female alike.

Presumption of cure, in column 3, was made on the general condition, as only very few cases would consent to confirmatory splenic puncture, and gland puncture (Kirk and Sati, 1940) was done only in later cases, of which only one presumed cure is included in this series. Of those in column 4, the majority completed one course of treatment but were not presumed cured. They refused, however, to stay in hospital for further treatment.

The mortality-rate is extremely high, and even in those who were discharged as presumed cures the survivor-rate was only 50 per cent., though some of these may have died from other causes.

Of the 12 survivors who were seen in January, 1940, the longest interval since discharge from hospital was 18 months and the shortest 8 months.

These results compare very unfavourably with those reported from India (Napier, 1932), where an 80 per cent. cure is the rule, but are in accordance with the experience of most workers in the kala-azar areas of the Sudan (cf. Horgan and Kirk, 1940).

It is impossible to get any reliable history from these people, but the impression gained from the more intelligent among them, and more particularly from two cases who developed what they said were the first signs of the disease while they were in hospital, was that it was an acute and, in some cases, fulminating type.

The following signs and symptoms were noted: epistaxis in 16 cases; persistent vomiting in 4 cases; dysenteric symptoms in 8 cases—two of these were infected with *Entamoeba histolytica*; pain and tenderness over the spleen in 15 cases; enlargement of liver in 21 cases.

Spleen puncture was persistently negative in three cases. Gland puncture was done only in five cases, and in two of these, who both had positive spleen puncture, L.D. bodies were not found. Thick blood-film examinations were done in every case, and in only two were L.D. bodies found.

Many cases had small crusted ulcers on legs and arms and sometimes on other parts of the body, but such ulcers are common in this part of the country. Scrapings were examined in many cases, but L.D. bodies were never found. Other skin lesions were not seen. Cancrum oris, a common and fatal complication, was seen in 18 cases, and of these 12 died.

TREATMENT

Standard treatment was with neostibosan, of which a course of about 4·8 gm. was given. This was usually followed, after an interval of 15–21 days, by a full course of tartar emetic, and in some cases further courses were given of one or other of these drugs or of anthiomaline.

In many of the cases even the immediate response to neostibosan was bad. This was particularly so in those cases who had cancrum oris. Ten cases with this complication, of whom three developed it after the commencement of treatment, were treated with neostibosan, and all of them died.

It was found that cases with this complication responded well to urea stibamine. Treatment was started with 0·05 gm. on two or three consecutive days, followe dby 0·1 gm. for a further three days, and two doses of 0·15 gm. every other day, increasing to 0·3 gm. as the maximum dose. Of eight cases with cancrum oris who were treated in this way only two died. In two of the cases that survived the complication had developed after treatment with neosti-

bosan had commenced. They were immediately switched over to the urea stibamine treatment, and the cancrum oris quickly cleared up.

General symptoms were also quickly brought under control by this method of treatment, high fever abated, the patients quickly put on weight, and the general condition improved.

SUMMARY

1. A brief account is given of an outbreak of kala-azar in a circumscribed area in the Upper Nile Province of the Anglo-Egyptian Sudan.

2. The relevant features of this outbreak were (*a*) the high proportion of cases in which the disease ran an acute course, (*b*) the frequency of serious complications such as cancrum oris, (*c*) the poor response to standard antimony treatment.

3. In cases with cancrum oris urea stibamine was found more efficacious than neostibosan.

ACKNOWLEDGEMENTS.—My thanks are due to the valuable assistance of Dr. E. S. Horgan and Dr. R. Kirk in the compilation of this paper, and to Dr. E. D. Pridie, Director of the Sudan Medical Service, for permission to publish it; also to Dr. R. M. Buchanan for the map on page 176.

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THE USE OF CERTAIN AROMATIC DIAMIDINES IN THE TREATMENT OF KALA-AZAR

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(*Received for publication September 7th*, 1940)

CONTENTS

I. INTRODUCTION

In a previous communication (Kirk and Sati, 1940*c*) it was stated that the authors had treated 28 cases of Sudan kala-azar with 4 : 4′-diamidino stilbene. Detailed histories were given of the first eight cases, which included two deaths and six recoveries. Points in connection with dosage, toxicity, etc., were discussed, and for this purpose reference was frequently made to observations on the remaining 20 cases, although at the time a number of these had not completed treatment and were still in hospital.

The treatment of these cases has now been completed, and those who have not died in hospital have all been discharged as provisionally cured. Only two further deaths have occurred since the first eight cases were reported, giving a total of four deaths in 28 cases, or an immediate-recovery rate of 86 per cent. It was intended to follow up those immediate recoveries over a period of some

months before publication, to estimate the possibilities of relapse, but this has become impossible in the meantime owing to the outbreak of hostilities in East Africa.

Clinical tests of two allied compounds—4 : 4′-diamidino diphenoxy propane and 4 : 4′-diamidino diphenoxy pentane (cf. Lourie and Yorke, 1939)—have also been made in human kala-azar. As the preliminary results with these compounds also are encouraging, they have been included in this paper.

With the exception of the first six recoveries, reported in our previous paper, it is to be regretted that in the present circumstances only immediate results can be recorded. These, however, are good, and in the opinion of the present writers compare favourably with the results previously obtained in this country with other methods of treatment. In addition, the first six recoveries have now been observed over a period of more than six months after cure, during which time no relapse has occurred.

II. CLINICAL MATERIAL

The observations recorded in the present paper have been made principally on patients coming to Singa hospital for treatment, all of whom presented the more typical clinical features of kala-azar—irregular fever, hepatic and splenic enlargement, anaemia and leucopenia. Diagnosis was confirmed in all cases by the demonstration of Leishmania in the splenic pulp or gland-juice (cf. Kirk and Sati, 1940*a*). It is interesting to note that the series includes three cases of typical clinical kala-azar, in which parasites were never found in the splenic pulp but were repeatedly found in the gland-juice, until finally they disappeared from this tissue also, as the signs and symptoms of the disease subsided under treatment.

With one exception, cases were accepted for treatment as they presented themselves to hospital, without any selection or discrimination. The series therefore includes moribund patients, admitted in the last stages of the disease, as well as ambulant cases who might be expected to make a good recovery. The one exception was a child with both kala-azar and myeloid leukaemia, who was excluded because attempts to assess the condition by repeated splenic punctures were liable only to accelerate the fatal termination.

More detailed analyses of the cases with reference to complications and to age- and sex-distribution are given later in the sections dealing with the separate groups treated by each drug.

III. GENERAL SCHEME OF TREATMENT

In addition to specific medication, certain general lines of treatment were followed which the writers have found of value in the treatment of kala-azar by any specific drug. In all cases particular attention was paid to the hygiene

of the mouth, and regular mouth-washes were used. With a view to combating the anaemia, patients were put on to an iron and arsenic mixture as routine, and were given a generous diet which included a daily ration of raw liver. Otherwise no special adjuvant treatment was given. Sodium pentonucleotide, which Zia and Forkner (1934) recommend in cases of severe leucopenia, was not available. Blood transfusion was not used, although it might have been a very valuable measure in some of the graver cases.

Concomitant infections and serious complications were treated as the occasion arose—malaria with quinine, amoebiasis with emetine, and pneumonia and septic complications with sulphapyridine supplemented where necessary by local measures. Surgical interference in cancrum oris was limited to the removal of necrotic tissue. Diarrhoea was treated symptomatically: usually it responded to a stock bismuth mixture, if this were given promptly and in sufficient dosage. Purgation, either as a routine measure at the outset of treatment or at any other time, was sedulously avoided. Epistaxis was sometimes a difficult problem, and was treated by orthodox measures applied locally to the nose, except on one occasion when these were supplemented by ascorbic acid, with doubtful result.

IV. CASES TREATED WITH 4:4′-DIAMIDINO STILBENE

The total number of cases treated with 4 : 4′-diamidino stilbene was 28. There were four deaths and 24 recoveries, giving an immediate-recovery rate of 86 per cent. Twenty-five per cent. of these immediate cures (six cases) have now been followed up over a period of 6–7 months, during which time there has been no relapse.

The following summary of the main clinical features in the 28 cases gives a general impression of the types of the disease on which the present observations are based.

The age- and sex-distribution of the 28 cases was as follows:

	Males	Females
1–4 years	1	1
5–9 years	3	3
10–19 years	7	—
20–29 years	7	—
30–39 years	6	—
40 years and over	—	—
Total	24	4

Haematology. In one case (no. 4 in the previous paper—Kirk and Sati, 1940*c*) the patient died before a blood examination was made, so that the figures given below refer to 27 cases only.

The red-blood count on admission was under 2 millions per c.mm. in 10 cases, between 2 and 3 millions in 14 cases, and between 3 and 4 millions in 3 cases. White-blood counts were as follows :

Under 2,000 in 8 cases
2,000–3,000 in 12 cases
3,000–4,000 in 5 cases
4,000–5,000 in 2 cases

In the routine differential count made on admission or shortly afterwards, eosinophils were absent in all cases except two.

Spleen. The size of the spleen on admission is expressed in the table below by the number of finger-breadths to which the organ extended below the costal margin.

Size of spleen	3	5	6	7	8	9	10	11
No. of cases	7	6	5	2	3	3	1	1

Complications. The clinical features and course of the disease were extremely variable, complications being frequent and often severe. Only in seven cases did the disease run an uncomplicated course. We have recorded epistaxis and diarrhoea as complications ; strictly speaking the former should probably be regarded rather as an integral part of the disease, but as a rule it was a sufficiently prominent symptom to attract special attention, and was sometimes alarming. In the case of diarrhoea, it was not possible to say whether in any instance it was due to kala-azar alone or to a superimposed bacterial infection, since facilities were not available for the routine examinations of stools by cultural methods.

Malaria was present as a complication in two cases, of which one died. Cancrum oris occurred in two cases, otitis media in one case, septic conditions affecting the mouth and face in two cases, and lobar pneumonia in four cases, one of which ended fatally. One case was complicated by amoebic dysentery, and severe diarrhoea occurred in six other cases. Epistaxis occurred in 10 cases, in five of which it was severe enough to give rise to anxiety. A febrile condition regarded as ' influenza,' which was prevalent at the time among the patients in the hospital and in the district, attacked a number of the cases, but was difficult to recognize since it was obscured by the symptoms of kala-azar unless these had largely disappeared under treatment before the influenza occurred.

Deaths. Case-histories of two of the four fatalities in this series have been given in the previous paper (Kirk and Sati, 1940*c*). They were two children,

aged 9 and 10 years, both admitted to hospital in a late stage of the disease. They died shortly after admission, having had a total of 125 mgm. and 75 mgm. 4 : 4′-diamidino stilbene respectively.

The third death occurred under similar circumstances in a youth aged 18 years, admitted to hospital in the last stages of exhaustion, with albumin, blood, pus and granular casts in the urine and a red-blood count under 2 millions per c.mm. He died with no particular symptoms, after having had four injections of 4 : 4′-diamidino stilbene, totalling 200 mgm.

The fourth death occurred in a little girl aged 4 years. On admission to hospital the disease was complicated by severe diarrhoea. Blood : R.B.C. 2,010,000, W.B.C. 3,800 ; differential count : P. 45 per cent. ; L. 43 per cent. ; L.M. 12 per cent. ; E. 0. She was given seven injections of 2 mgm. per kilo. over a period of 14 days. The diarrhoea stopped on the third day, and the patient appeared to be progressing well, when she suddenly died, for no apparent reason, on the day following the seventh injection.

Hospitalization. The average period of hospitalization in the 24 successful cases was 16 weeks, the longest period being 27 weeks and the shortest 5 weeks. As a rule, the time spent in hospital included observation-periods of 3–4 weeks, during which no treatment was given.

V. NOTE ON AN ANTIMONY-RESISTANT CASE

The following history is of particular interest, since it refers to a relapsed case which was apparently resistant to antimony but reacted rapidly to 4 : 4′-diamidino stilbene. The case was seen by one of us in March, 1940, but the treatment was carried out by Dr. R. T. Campbell in Gedaref hospital. We were unable to see this case again and circumstances have prevented a subsequent follow-up, but Dr. Campbell has very kindly placed at our disposal such information as is available. The following extract from a letter received from Dr. Campbell on May 12th, 1940, describes the progress of the case under treatment :

'. . . that soldier whom you saw in the military hospital. He had been in hospital since the middle of September last, and had had two courses of neostibosan and three of antimony tartrate with no result, i.e., spleen remained much enlarged and strongly positive for L.D. bodies. On March 20th I started him on daily injections of one ampoule [i.e., 100 mgm. 4 : 4′-diamidino stilbene] in 10 c.cm. of distilled water. His spleen gradually diminished in size, and all pain left him. On April 10th his spleen was just palpable, and spleen puncture negative. On April 14th, on completion of course of 24 injections, spleen had shrunk even more, and spleen puncture, got with difficulty, was negative again. So he has been discharged as apparently cured. However, as he is in the Eastern Arab Corps we will be able to keep an eye on him and see if he remains negative.'

After discharge from hospital the patient went on leave and then rejoined his unit, at present on active service. Owing to war conditions it has not been possible to follow up the case in the meantime, but as he is a soldier the presumption is that if relapse occurred he would be sent back to hospital, and thus come under observation once again.

VI. CASES TREATED WITH 4:4′-DIAMIDINO DIPHENOXY PENTANE

The number of cases treated with 4 : 4′-diamidino diphenoxy pentane was 13. There were three deaths, nine immediate recoveries and one doubtful recovery. The latter was a small boy, aged 6, who ran away from hospital before his condition was considered entirely satisfactory. Leishmania had disappeared from spleen and glands as estimated by three punctures, but there was still some irregular fever, the spleen was unchanged and further improvement in the blood picture was desirable. If this case is excluded from the series, the immediate-recovery rate is 75 per cent.

The age- and sex-distribution of the 13 cases was as follows :

	Males	Females
1–4 years	—	1
5–9 years	2	1
10–19 years	1	—
20–29 years	3	1
30–39 years	3	—
40–49 years	—	—
Over 50 years	1	—
Total	10	3

Haematology. One patient, who was admitted in a moribund condition with lobar pneumonia as well as kala-azar, died before blood examination was made, so that the figures given below refer to 12 cases only.

The red-blood count on admission was under 2 millions per c.mm. in one case only, between 2 and 3 millions in 5 cases, and between 3 and 4 millions in 6 cases. White-blood counts were as follows :

Under 2,000 in 4 cases
2,000–3,000 in 2 cases
3,000–4,000 in 2 cases
4,000–5,000 in 2 cases
5,000–6,000 in 1 case
6,000–7,000 in 1 case

In the routine differential count made on admission or shortly afterwards, eosinophils were absent in 11 of the 12 cases.

Spleen. The size of the spleen on admission is given below in finger-breadths.

Size of spleen	2	4	6	8	9
No. of cases	2	1	4	2	4

Complications. This group also presented a number of complications in addition to the usual clinical findings. Only one case ran an uncomplicated course from beginning to end. Two cases, one of which died, were complicated by lobar pneumonia. Cancrum oris occurred in one case, which survived, although the patient had in addition otitis media and severe diarrhoea. Severe diarrhoea occurred in four cases, in two of which it resulted in a fatal termination. Otitis media occurred in five cases, tonsillitis in one case, and epistaxis in six cases, in two of which it was severe. ' Influenza ' undoubtedly attacked a number of the cases.

Deaths. The first death occurred in a man of 35 years who was brought to hospital with kala-azar complicated by lobar pneumonia of unknown duration. On admission he was only semi-conscious and was in an extreme state of exhaustion. He remained in this state until he died six days later. During this time he was given four injections of the drug, totalling 200 mgm.

The second death occurred in unfortunate circumstances. The patient was a man of 38 years, a fairly average case of kala-azar with no unusual features. After five weeks' treatment the fever settled, and he appeared to be making an excellent recovery in every way. After being afebrile for nearly three weeks he went out one day and drank a great deal of merissa (native beer), returning to the hospital at night very intoxicated. The following day he developed very severe diarrhoea, which resisted every form of treatment and ended fatally in less than 48 hours.

The third fatality occurred in a man of 55 years. His spleen on admission was enlarged to 9 finger-breadths below the costal margin, and the liver was palpable. The urine contained albumin, epithelial cells, hyaline and granular casts. Blood : R.B.C. 3,040,000, W.B.C. 7,000 ; differential count : P. 64 per cent. ; L. 31 per cent. ; L.M. 5 per cent. ; E. 0. There was a history of diarrhoea before admission, but microscopical examination of the stool revealed no blood, mucus, ova or protozoa, and there was no diarrhoea at the time of admission on March 11th, 1940. Between March 13th and April 8th he was given 14 injections of 1·2 mgm. per kilo. On April 6th he suddenly developed very severe diarrhoea which resisted every form of treatment. Three days later he became collapsed and passed into a semi-conscious condition. He died on April 10th, having had a total dosage of 1·12 gm. of the drug.

Hospitalization. The average period of hospitalization in successful cases was 14½ weeks, the shortest period being 12 weeks and the longest 18 weeks.

VII. CASES TREATED WITH 4:4′-DIAMIDINO DIPHENOXY PROPANE

Only two cases were treated with 4 : 4′-diamidino diphenoxy propane. One, which also had lobar pneumonia, recovered, and the other died. The case-histories are as follows.

CASE A

Male, aged 20, Bargawi tribe, admitted on February 24th, 1940, after a traumatic wound. General condition weak, fevered. Weight 6 stone, 9 lb. Spleen palpable 3 finger-breadths, liver 2 finger-breadths. Blood: R.B.C. 2,450,000, W.B.C. 3,200; differential count: P. 40 per cent.; L. 56 per cent.; L.M. 4 per cent.; E. 0. Spleen and glands were positive for Leishmania on March 9th.

From March 11th to May 11th he was given 30 injections of 80 mgm. (1·9 mgm. per kilo. body weight) of 4 : 4′-diamidino diphenoxy propane on alternate days. On March 16th he developed a left-sided lobar pneumonia, and for the following week was most of the time in a delirious condition. From this he passed into a 'typhoid state,' in which he remained for another week. In the meantime the injections were continued, although the patient was expected to die at any moment. He remained in a very exhausted condition until April 21st, when the fever subsided.

By May 15th he had been afebrile for three weeks and showed signs of general improvement. The course of 30 injections had been completed, the spleen and liver had become unpalpable, but Leishmania were found in the gland-juice. From May 22nd to June 3rd a course of 15 daily injections of 80 mgm. was given. There was no subsequent rise of fever. Gland puncture was negative on May 28th, June 11th and June 17th. Sternal and spleen puncture were also both negative, the latter being done through an intercostal space over the area of splenic dulness. Blood (on June 20th): R.B.C. 4,020,000, W.B.C. 7,400; differential count: P. 49 per cent.; L. 45 per cent.; L.M. 4 per cent.; E. 2 per cent.

He was discharged on June 21st. The period spent in hospital was 17 weeks, during which he had gained 1 stone in weight and received a total dosage of 3·52 gm. of 4 : 4′-diamidino diphenoxy propane.

CASE B

Male, aged 30, Fellata race, a refugee from Abyssinia, admitted on March 8th, 1940, in an extremely dirty, verminous and exhausted condition. Weight 5 stone. Spleen and liver both palpable 1 finger-breadth below the costal margin. Leishmania were found in spleen and glands.

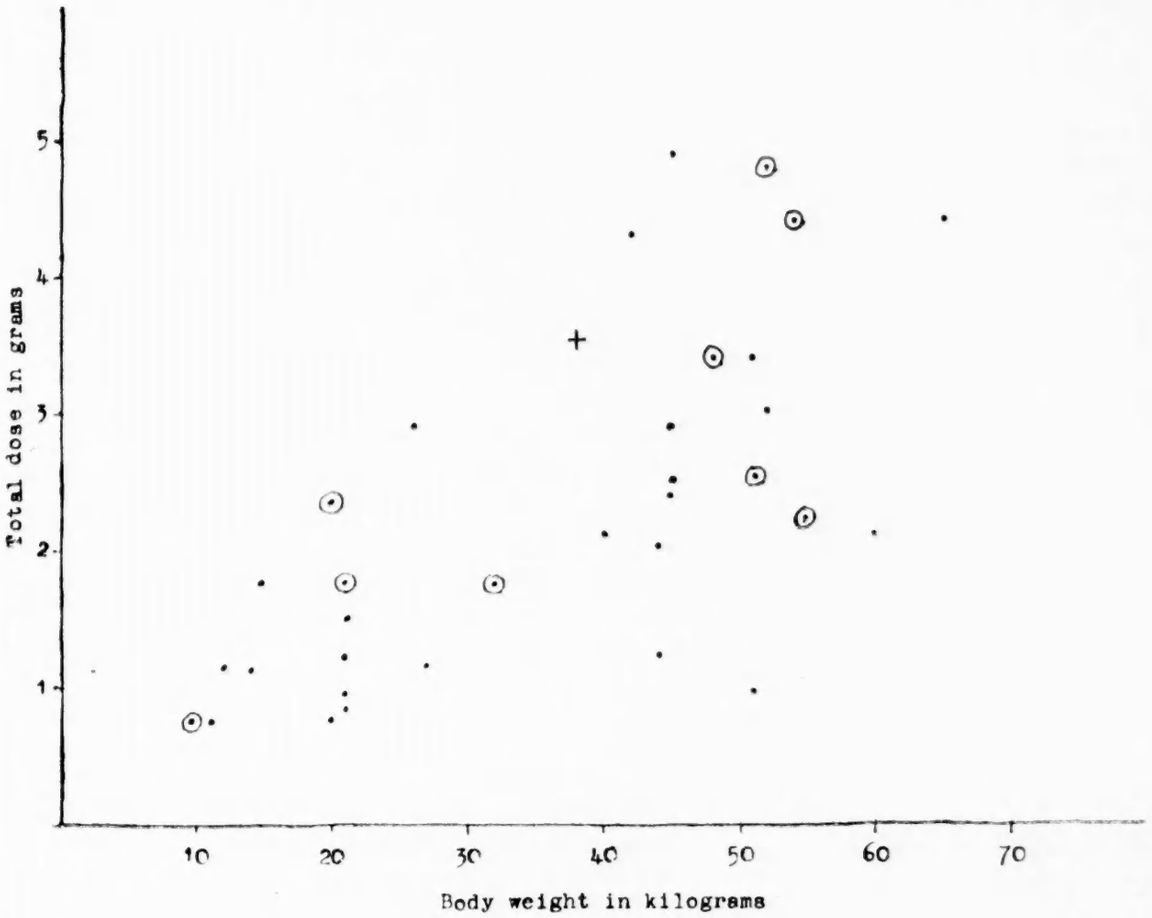
He was given four injections of 80 mgm. (2·5 mgm. per kilo.) on alternate days between March 10th and March 18th, when he developed severe haemorrhage from the gums pharynx and fauces, which proved fatal.

VIII. ADMINISTRATION AND DOSAGE

Intravenous injections. With the exception of one case, the drugs were given by the intravenous route, freshly dissolved in distilled water in a concentration of 10 mgm. per c.cm. 4 : 4′-diamidino diphenoxy pentane dissolves readily in higher concentrations and can be given intramuscularly; but this causes some discomfort, and adults to whom we gave a few injections by the intramuscular route informed us that they preferred to have the remainder of their treatment intravenously.

Case treated by intramuscular injections. The one exception was a child aged 18 months. Suitable veins were difficult to find in this patient, and the whole treatment from start to finish was given by intramuscular injections of 4 : 4′-diamidino diphenoxy pentane into the buttocks. No untoward effects resulted from these injections, and the ultimate cure was eminently satisfactory from every aspect. No attempts were made to give either 4 : 4′-diamidino stilbene or 4 : 4′-diamidino diphenoxy propane by the intramuscular route.

Schemes of treatment. The dose per injection varied from 1 to 2·6 mgm. per kilo. body weight, and those quantities were well tolerated, even over long courses of treatment. Following the experience of Adams and Yorke (1939), treatment was carried out in the first cases on 4 : 4′-diamidino stilbene by short courses of 8–10 daily injections, separated by longer periods, during which the clinical improvement following each course was observed. It appeared from the observations on the early cases that approximately 30 injections were required



Scattergraph showing body weight and total dosage required for cure in cases of kala-azar treated with aromatic diamidines.

· denotes cases treated with 4 : 4′-diamidino stilbene.
⊙ ,, ,, ,, ,, 4 : 4′-diamidino diphenoxy pentane.
\+ ,, case ,, ,, 4 : 4′-diamidino diphenoxy propane.

before cure might reasonably be expected in the average case. Later cases on both diamidino stilbene and diphenoxy pentane were therefore treated by long continuous courses of 20–30 injections given on alternate days instead of daily. It was difficult to show that any advantage was gained by this method, as Leishmania were still present in a number of cases even after 30 injections ; so a more intensive form of treatment was adopted. Courses of 15 daily

injections (2 mgm. per kilo.) were used, separated by shorter intervals of seven days. This did seem more effective. Only three cases were from the outset treated entirely on this scheme. One of these cases was complicated by lobar pneumonia, but all three were discharged as cured after five weeks' hospitalization only, as compared with an average of 15 weeks' in the whole series. This, of course, may be entirely fortuitous, since only three cases are concerned. Cases vary greatly in their reaction to treatment, and these three may have been fortunate ones.

The total dosage required for cure varied within wide limits with different cases. The largest quantity required in any case was 4·9 gm., the smallest 0·75 gm. These figures do not take body weight into account, but the diagram on page 189 indicates that there is no constant relation between body weight and the total quantity required for cure. Other factors, such as the type, duration and severity of the disease, are probably of more importance in this connection.

If the total dosage is expressed in milligrams per kilo. body weight, wide variations are still evident in the amounts of drug required in different cases. The largest total dosage, expressed in this way, was 111 mgm. per kilo., given over a period of 21 weeks to a boy 12 years old, in whom the disease was complicated by cancrum oris and amoebic dysentery. These complications cleared up satisfactorily at an early stage, but Leishmania persisted in the spleen and lymphatic glands, and the prolonged treatment was necessitated, not by the complications, but in order to eradicate the parasites. The smallest total dosage, 19 mgm. per kilo., over a period of five months, was given to a man aged 28, whose progress has already been described in some detail as case no. 7 in the previous paper (Kirk and Sati, 1940*c*).

IX. TOXICITY

On two occasions intravenous injections of 4 : 4′-diamidino diphenoxy pentane were immediately succeeded by rather alarming symptoms—syncopal attacks, with temporary loss of consciousness and epileptiform twitching. In one instance the symptoms occurred with an initial injection of 100 mgm.; the dose was reduced to 80 mgm. for the succeeding four injections, and thereafter increased once more to 100 mgm., without any further reactions supervening. In the second instance the symptoms occurred after an injection of 100 mgm. in a patient who had already had 10 injections of this dose without any reaction. Subsequent injections were continued without alteration in the dosage, and no further reactions occurred. In both instances the symptoms were very transient and passed off in a few seconds.

Apart from the reactions in these two cases, there is little to add to the remarks included under this heading in our previous paper (Kirk and Sati, 1940*c*), where a number of minor toxic reactions were recorded. These were breathlessness, dizziness, epigastric discomfort and vomiting—all transient

symptoms, passing off in a few moments. Such symptoms were not taken as an indication to withhold further injections. One of the best recoveries in the series was a case of lobar pneumonia and kala-azar, treated by two intensive courses of 15 daily injections of 100 mgm. (2 mgm. per kilo.) of 4 : 4′-diamidino stilbene. Vomiting occurred with the first four injections, but daily injections were continued with no reduction in dose. After the fourth injection no more vomiting occurred, and the patient was discharged as cured in five weeks. There has been no further indication of any toxic action on the kidney with the doses employed. Indeed the comparative absence of severe toxic symptoms of any kind has been noteworthy, even with prolonged treatment.

One case in the present series died rather unexpectedly for no very apparent cause. In the past, sudden death without any apparent reason has been one of the chief bugbears in the treatment of kala-azar by antimony. Mainzer and Krause (1940) have shown that tartar emetic in therapeutic doses produces pathological changes in the electrocardiogram, and suggest that those sudden deaths are due to the toxic action of antimony on the heart-muscle. It has always been noted in the Sudan that sudden deaths in the course of antimony treatment were much commoner in kala-azar cases than in cases of schistosomiasis, possibly owing to the more debilitated condition of the heart-muscle in the former condition. There is no evidence that the sudden death we have described was in any way associated with a toxic effect of the drug used, but, owing to the importance of such accidents in the past with other methods of treatment, this is a matter for further observation.

X. GENERAL COURSE OF THE DISEASE DURING TREATMENT

Frequently the first indications of progress were reduction of the fever and improvement in the general condition, usually within the first week or two. At this stage the spleen and blood picture were unchanged, and parasites were still readily demonstrated. This occurred in 19 cases. In the remainder of the series the fever persisted over several weeks before finally subsiding as the other symptoms improved. Of those 19 cases, two were treated with diphenoxy pentane; in these there was no subsequent recurrence of the fever during the period of treatment. The other 17 were treated by diamidino stilbene, and in all of them the fever recurred subsequently, sometimes with a temporary exacerbation of the other signs of the disease. How far this difference is the result of varying schemes of dosage it is difficult to say. The two cases on diphenoxy pentane were treated by courses prolonged over 40–60 days, during which injections were given on alternate days only, whereas those on diamidino stilbene were given intensive short courses of 8–16 daily injections separated by intervals without treatment.

In a number of instances the injections appeared to cause exacerbations

of fever. This was particularly noticeable when a new course of injections was started in cases where the temperature had been normal for some time; also in cases treated by diphenoxy pentane where injections were given on alternate days. In the latter a sharp access of fever on the days of the injection, with remissions on the intervening days, produced temperature-charts exactly simulating tertian malaria, which had to be excluded by repeated blood examinations. As a rule, these febrile reactions occurred when there was some evidence that the disease was still active. Towards the completion of treatment, when Leishmania had disappeared and patients might possibly be cured, or nearly so, the injections had little or no effect on the temperature.

Shrinkage of the splenic tumour occurred in all except four cases, but the manner of its occurrence was extremely variable. Sometimes the first effect of treatment was to produce a noticeable enlargement of the spleen, which might be accompanied by an aggravation of the fever. Minor temporary fluctuations in the size of the organ were very common during the course of treatment. In a few cases shrinkage began at an early stage of the treatment and continued slowly and progressively. More commonly there was little change in the size of the spleen, apart from minor temporary fluctuations, over a long period, during which the fever, blood picture and general condition improved. Then towards the end of treatment the organ would suddenly begin to shrink, and a comparatively large tumour might subside rapidly in the course of a week. Complete disappearance of the splenic tumour is difficult to obtain in long-standing cases, where fibrotic changes may have taken place. It was obtained in under 40 per cent. of cases in the present series. This is in agreement with the experience of Henderson (1937), who records also that in cases discharged with a slightly palpable spleen further shrinkage often occurred later, and the organ was frequently unpalpable six months after discharge.

Taking the present series as a whole, 70 per cent. of cases were discharged with unpalpable (34 per cent.) or slightly palpable spleens (36 per cent.). In 20 per cent. the spleen was still palpable 2–5 finger-breadths below the costal margin at the time of discharge. In these cases, however, a very considerable reduction had occurred during treatment. In the remaining 10 per cent. (4 cases) there was no appreciable alteration in the size of the spleen, which was of stony hardness but negative for Leishmania on repeated punctures.

Improvement in the blood picture was usually more rapid in the case of the red cells than in the case of the leucocytes, but the latter have probably more prognostic significance. According to Napier's (1932*b*) experience in India, no case relapsed in which the total white count at the end of treatment was over 6,000 per c.mm., though only 25 per cent. of the cases below this figure did so. In the present series, 11 cases treated with 4 : 4′-diamidino stilbene and 3 cases treated with 4 : 4′-diamidino diphenoxy pentane were discharged as provisionally cured with the total white count still under 6,000 per c.mm., although it had improved considerably under treatment. Five of these cases fall into the

early group of recoveries, which have now been followed up over a period of 6–7 months. Not only has no relapse occurred, but in some instances a moderate leucocytosis developed some months after discharge from hospital. In the differential count the eosinophils have some significance (Archibald, 1923). Diminution of these is a constant feature of Sudan kala-azar, indicated usually by a complete absence of eosinophils in the routine blood counts. Their reappearance, even in normal proportions, is a useful prognostic sign, and was observed with remarkable constancy in the present series.

Patients were weighed at weekly intervals, and in a majority there was an increase in weight during treatment varying from 3 to 30 lb. In others there was no change. Minor fluctuations in weight were often associated with the vicissitudes which occurred during the course of the disease, but in no case was the weight at the end of treatment less than it had been on admission. Improvement in general condition was not necessarily accompanied by increase in weight, and where discrepancy between the two occurred the former appeared to be the more valuable indication of progress.

In 32 per cent. of the cases skin rashes appeared towards the end of treatment. These varied in appearance from inconspicuous finely punctate rashes to frankly nodular eruptions. They closely resemble the skin rashes often seen in cases of Sudan kala-azar which are progressing favourably under treatment with antimony. An account of these eruptions, including illustrations from the present series, has been published elsewhere (Kirk and Sati, 1940*b*), and it has been suggested that their appearance during the course of treatment has a good prognostic significance as regards cure of the visceral disease. Leishmania were demonstrated in the eruption in one instance only in the present series.

XI. CRITERIA OF CURE

According to Findlay (1939), the best evidence of cure in kala-azar is an entire absence of clinical symptoms for a period of six months after the termination of treatment. The first six recoveries have now satisfied this criterion. In the case of immediate results it is often difficult to estimate when a patient under treatment can be regarded as cured, since there are at present no clinical or laboratory methods which can do more than indicate the probability of successful cure at this stage. In the present series progress under treatment was estimated by the following criteria: disappearance of fever, improvement in general condition, diminution of splenic tumour, return of the blood picture to normal, and disappearance of the parasites from the tissues. Little reliance can be placed in any one of these criteria without reference to the others, but taken together they enable a fairly accurate estimate of progress to be made.

Before being discharged as cured, patients were afebrile for periods varying from one to three months, during which it was difficult to enforce hospital discipline and most of the patients lived largely as they pleased, often regarding the hospital merely as a place where food was available and a bed prepared for

them at night if they chose to make use of it. During this period several consecutive negative spleen and gland punctures were required. In the case of the first six recoveries these examinations have already been recorded. Cultural methods are hardly feasible in Sudan out-stations, so reliance had to be placed on the microscopical examination of stained smears to determine the presence or absence of parasites. For this reason, a somewhat stricter routine was instituted in the later cases as compared with the first six. At least three negative spleen punctures and three negative gland punctures were required. Sometimes as many as six negative punctures were recorded in cases where there was a lag in recovery as judged by the other criteria. In a few cases only it was not possible to obtain a third spleen puncture owing to the rapid and complete disappearance of the splenic tumour. Except in children, sternal puncture was carried out also as a final measure before discharge, and this always confirmed the negative findings in spleen and glands. In three cases Leishmania were found in small cutaneous ulcers on admission. At discharge these ulcers were negative for Leishmania and progressing to scar-formation.

The course of improvement in the fever, general condition, splenic tumour and blood picture have been described in some detail in the previous section.

XII. COMMENTS

Yorke (1940) has pointed out that these aromatic diamidines are of entirely different chemical constitution from any known therapeutic substance. In the 43 cases of kala-azar under the care of the present writers, treatment has been entirely dependent on the action of one or other of the aromatic diamidines. No other specific against leishmaniasis has been used, and any adjuvant treatment which has been employed would not, in itself, be likely to have any effect on the ultimate prognosis. The results show clearly and unequivocally that these compounds are therapeutically effective in human kala-azar. A purist might object that the results are unconvincing in the case of 4 : 4′-diamidino diphenoxy propane, where only two cases were treated, with one recovery and one death. This criticism, however, cannot be applied to the series treated with 4 : 4′-diamidino stilbene, where an 86 per cent. recovery-rate was obtained among 28 cases.

Including the case treated by Dr. Campbell in Gedaref, a total of 44 patients have been treated with the three drugs. There have been eight deaths, giving a mortality-rate of 18 per cent. Analysis of the deaths shows that five of the eight occurred in patients brought to hospital *in extremis* or in an advanced state of illness and exhaustion. Some of these cases might possibly have been saved by the addition of blood transfusion to the adjuvant methods of treatment, but it is unlikely that any specific drug alone will produce 100 per cent. recovery in a series including patients in whom the disease is already in its final stage at the beginning of treatment. The case that died after a drinking-bout is in a different category, but it may be mentioned that other patients also indulged

in a certain amount of riotous living during convalescence, with less disastrous consequences.

There are wide differences in the mortality-rates of the three groups treated by the different drugs, the figures being 14 per cent. in the case of 4 : 4′-diamidino stilbene, 23 per cent. with 4 : 4′-diamidino diphenoxy pentane, and 50 per cent. with 4 : 4′-diamidino diphenoxy propane. It might be presumed at first sight that the drugs are not all equally effective, or differ in toxicity, but the numbers are too small for comparisons. In the case of 4 : 4′-diamidino diphenoxy propane, for example, one death makes the difference between 100 per cent. recovery and a 50 per cent. mortality-rate. In view of this, and of the great variation of individual cases in severity, no conclusions as to differences in effectiveness between the three drugs can be drawn from these figures.

According to Napier (1932*a*), the difference between antimony tartrate and the pentavalent compounds in the treatment of kala-azar 'is so striking that it does not require more than a small series of cases treated by each class of drug for its demonstration,' but when it 'comes to differentiating between the various pentavalent compounds the case is very different. For this purpose reference to the ordinary hospital or dispensary records of treatment is quite valueless, not only because the diagnosis is seldom confirmed by demonstrating of the parasite, but because different physicians adopt not only different schemes of dosage, but different standards for judging whether a patient is cured or not.' Attempts to compare the results in the present paper with the published results of treatment by other methods are beset by the same difficulties, and an additional fallacy is introduced when a comparison is made with the results which have been obtained in other countries. The present results compare unfavourably with those which have been reported in the case of Indian kala-azar, especially as regards the prolonged hospitalization. It has been pointed out elsewhere (Kirk and Sati, 1940*c*) that comparison with Indian results is no criterion by which the value of a new remedy in Sudan kala-azar should be assessed. Compared with the results previously obtained by other methods of treatment in the Sudan, as recorded by Christopherson (1917), Archibald (1923), Henderson (1937), Stephenson (1940) and in the annual reports of the Sudan Medical Service, the present results are good.

As the object of the present work was primarily to determine whether the new compounds could cure kala-azar, each case was treated by one drug only. No attempt has been made to combine the aromatic diamidines in treatment with antimonials, although such a combination has interesting possibilities. There is some theoretical ground for the view that the new compounds may possibly act in a different way from the antimonials. It was shown by Noguchi (1924) that tartar emetic has very little effect on cultures of Leishmania. Napier (cf. Boyd, Napier and Roy, 1931) records similar findings with the pentavalent compounds, and concludes that the action of antimony on the parasites is an

indirect one—a view expressed some years earlier by Acton and Chopra (1927), who concluded that several factors were concerned in the action of antimony in kala-azar. The therapeutic application of the aromatic diamidines, on the other hand, was undertaken as a result of experimental observations (Yorke, 1940) on their trypanocidal activity *in vitro*, and Adler and Tchernomoretz (1939) have shown that 4 : 4′-diamidino stilbene is four times as toxic to cultures of Leishmania as tartar emetic, and at least 100 times as toxic as neostibosan. In this connection the history of Dr. Campbell's case is of special interest, since in this case the change from one drug to the other produced a very rapid reaction to treatment. This patient was discharged as apparently cured after only 24 days' treatment with 4 : 4′-diamidino stilbene, involving a total dosage of 2·4 gm. of the drug. Whether the rapidity of this recovery was due to the previous treatment with antimony cannot be stated at present, but further observations on this point are obviously desirable.

XIII. SUMMARY

1. The therapeutic action of three aromatic diamidines—4 : 4′-diamidino stilbene, 4 : 4′-diamidino diphenoxy pentane, and 4 : 4′-diamidino diphenoxy propane—has been tested clinically in 44 cases of Sudan kala-azar, and the results compare favourably with those previously obtained in the Sudan with other methods of treatment.

2. In 28 cases treated by 4 : 4′-diamidino stilbene the immediate-recovery rate was 86 per cent. Twenty-five per cent. of these immediate cures have been followed up over a period of 6–7 months, during which time there has been no relapse.

3. In 13 cases treated by 4 : 4′-diamidino diphenoxy pentane the immediate-recovery rate was 75 per cent.

4. Two cases only were treated by 4 : 4′-diamidino diphenoxy propane, with one death.

5. Notes are given of a case which was apparently resistant to antimony but improved rapidly when the treatment was changed to 4 : 4′-diamidino stilbene.

6. Treatment was carried out by intravenous injections, except in one case where intramuscular injections of 4 : 4′-diamidino diphenoxy pentane were used.

7. The dose given at each injection varied from 1–2·6 mgm. of the drug per kilo. body weight.

8. The total dosage required for cure varied considerably with different cases.

9. In doses which are therapeutically effective, the toxicity of these compounds is low, even with prolonged courses of treatment.

ACKNOWLEDGEMENTS.—We are deeply indebted to Professor Warrington Yorke, for his kind advice and guidance during the whole course of this work, and for supplies of the drugs with which it was carried out; also to Dr. R. T. Campbell, for particulars of the antimony-resistant case; to Dr. E. D. Pridie, Dr. E. S. Horgan and Dr. N. L. Corkill, for help and facilities to carry out the work; and to the Director of the Sudan Medical Service for permission to publish these observations.

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[Since this paper went to press, a letter, dated September 25th, 1940, has been received from Dr. Kirk stating that two espundias have been discharged as possible cures, as have also two further antimony-resistant and antimony-relapsed cases, who reacted very well to 4 : 4′-diamidino stilbene. —EDITORS.]

THE ACTION OF 4:4′-DIAMIDINO STILBENE ON VARIOUS PIROPLASMS

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Professor Warrington Yorke, F.R.S., kindly sent us samples of 4 : 4′-diamidino stilbene for testing on piroplasms, and informed us that he and Dr. Lourie had found it effective in cases of *Babesia canis* in puppies.

We tested the drug on the following piroplasms : *Theileria annulata* in calves ; *Anaplasma marginale* in calves ; *Anaplasma ovis* in goats ; *Babesiella ovis* in goats ; *Babesia bigemina* in calves.

In addition, Dr. M. Sturman, veterinary surgeon of the Haklait Cattle Insurance Company, tested the drug in the field on theileriosis.

Without going into details of individual experiments, we may say that both in the field and in the laboratory the drug in doses up to 10 mgm. per kilo. body weight had no effect whatever on the clinical course, on Koch bodies in the lymphatic glands or on parasites in the blood in six severe cases of *Theileria annulata*.

In the case of *Anaplasma marginale* and *Anaplasma ovis* we worked with splenectomized calves and goats respectively, in order to eliminate the natural defences of animals against these parasites (very marked in the case of *A. marginale* in calves), and thus to determine any action of the drug on the parasites without complicating factors. The drug in doses up to 10 mgm. per kilo. body weight had not the slightest effect on anaplasmosis in eight goats and ten calves.

Babesiella ovis. The drug was found to have a very marked therapeutic effect on *Babesiella ovis* in goats. Therapy should be commenced as quickly as possible, because the clinical condition is not always a guide to prognosis ; with our strain an infected animal, apparently in good condition one day, may be found dead on the following day without having previously shown alarming premonitory symptoms.

METHODS

In order to exclude the natural defences against the parasite, we used only splenectomized goats. Almost all adult goats in Jerusalem have a latent infection of *Anaplasma ovis* which is revealed by splenectomy. The infection with *Anaplasma* produces a severe anaemia in splenectomized goats, usually

non-fatal. In several instances we noted inhibition of *B. ovis* by rapidly multiplying *A. ovis*. We therefore limit our observations to splenectomized animals in which the infection with *Anaplasma* was on the downgrade and did not involve more than 20 per cent. of the total red cells. *B. ovis* produces a fatal infection in such animals, which are therefore satisfactory for our purpose.

In the majority of experiments with *Babesiella ovis* and *Babesia bigemina* we determined approximately the number of infected red cells per c.cm. by counting the total number of leucocytes in a haemocytometer and then comparing the number of parasites to the number of leucocytes in a thick drop. All injections of the drug were made intravenously.

BABESIELLA OVIS IN GOATS

The history of the strain employed is as follows.

Goat No. 3

May 19th, 1939. Animal splenectomized.

„ 25th. *Babesiella ovis* were found in blood smears. Temperature 41·3° C. The parasites increased, and the animal died of severe anaemia on May 28th. The strain was maintained by direct passage through goats.

Goat No. 2

April 16th, 1939. Animal splenectomized.

„ 20th. *A. ovis* appeared and were present until September 20th.

May 11th. 25 per cent. of the red cells were infected with *Anaplasma*. Weight 30 kilo. Received 300 mgm. of 4 : 4′-diamidino stilbene. There was no effect on the *Anaplasma*.

May 20th. Received 20 c.cm. blood from goat no. 3. *B. ovis* were too few to be revealed in smears, but were present in the blood, as the following experiment shows.

Goat No. 4

May 19th, 1939. Animal splenectomized and developed a slight infection of *A. ovis*.

June 6th. 50 c.cm. blood from goat no. 2 injected into goat no. 4.

„ 13th. Temperature 40·3° C. *B. ovis* found in smears.

„ 15th. Temperature 41·6° C. Red cells 10×10^6 per c.mm.

„ 16th. Temperature 41·5° C. Red cells 7×10^6 per c.mm.; 10 infected red cells per field.

June 17th. Animal in distress and refused food. Red cells 7×10^6 per c.mm. Weight 27 kilo. 67·5 mgm. 4 : 4′-diamidino stilbene injected.

June 18th. No parasites found. The animal made an uneventful recovery.

GOAT No. 7

June 4th, 1939. 85 c.cm. blood from goat no. 2 were injected into goat no. 7. During an observation-period of one month no parasites were found in the blood.

July 3rd. Animal splenectomized.

„ 19th. *Anaplasma* appeared; 8 per cent. of the red cells infected.

„ 21st. 20 per cent. of the red cells infected.

August 1st. *B. ovis* numerous in the blood.

„ 4th. 15×10^6 red cells per c.cm. infected with *B. ovis*. Weight 25 kilo. 100 mgm. of the drug was injected.

August 5th. No *B. ovis* found in smears, but the animal died.

GOAT No. 9

June 22nd, 1939. Animal splenectomized. It developed anaplasmosis and a severe anaemia, the red cells falling from 15×10^6 to 8×10^6 per c.mm. by August 11th, after which date the *Anaplasma* diminished.

August 1st. Received 60 c.cm. blood from goat no. 7.

„ 28th. Temperature 41° C. 8 per cent. of the red cells infected with *Anaplasma*. 6×10^6 red cells per c.cm. infected with *B. ovis*. Weight 16 kilo. Animal received 50 mgm. of the drug.

August 29th. No *B. ovis* found. The animal made an uneventful recovery. 50 mgm. of the drug destroyed the parasites in approximately $7{,}500 \times 10^6$ red cells within 24 hours.

GOAT No. 11

July 31st, 1939. Animal splenectomized.

August 4th. 20 per cent. of the red cells infected with *Anaplasma ovis*.

„ 6th. Received 40 c.cm. blood from goat no. 7.

„ 9th. 9×10^6 red cells per c.cm. infected with *B. ovis*.

„ 10th. 20×10^6 red cells per c.cm. infected with *B. ovis*. Weight 15 kilo. Received 50 mgm. of the drug.

August 11th. No *B. ovis* found. 20 per cent. of the red cells infected with *Anaplasma*. The animal made an uneventful recovery. 50 mgm. of the drug destroyed the parasites in approximately $24{,}000 \times 10^6$ red cells within 24 hours.

BABESIA BIGEMINA IN CALVES

For reasons of economy, we restricted our experiments to calves. Since normal calves are often cured spontaneously, we used splenectomized animals. Again for reasons of economy, we generally used the same animal for experiments on *Theileria annulata*, *B. bigemina* and *A. marginale*.

CALF NO. 7

May 3rd, 1939. Animal splenectomized.

June 20th. Inoculated with *T. annulata.*

,, 28th. Inoculated with 100 c.cm. blood from a cow naturally infected with *B. bigemina.*

July 3rd. *B. bigemina*, *A. marginale* and *T. annulata* found in smears.

,, 4th. 3×10^6 red cells per c.cm. infected with *B. bigemina.*

,, 6th. 11×10^6 red cells per c.cm. infected with *B. bigemina.* Weight 30 kilo. Received 90 mgm. of 4:4′-diamidino stilbene.

July 7th. No *B. bigemina* found. *A. marginale* and *T. annulata* not affected. 90 mgm. of the drug destroyed the parasites in approximately $25{,}300 \times 10^6$ infected red cells.

July 9th. The animal died with a mixed infection of *T. annulata* and *A. marginale.*

CALF NO. 5

March 28th, 1939. Animal splenectomized (this animal had a previous infection of *T. annulata*, and parasites were constantly present in the blood).

July 5th. Received 40 c.cm. blood from calf no. 7.

,, 10th. *B. bigemina* appeared in the blood.

,, 11th. 17×10^6 red cells per c.cm. were infected. Weight 50 kilo. Received 200 mgm. of the drug.

July 12th. No *Babesia* found. 200 mgm. of the drug destroyed the parasites in approximately $65{,}000 \times 10^6$ infected red cells within 18 hours.

July 18th. *A. marginale* appeared.

,, 22nd. *B. bigemina* reappeared.

,, 27th. 12×10^6 red cells per c.cm. were infected. 1 p.m.: 200 mgm. of the drug administered. 2.30 p.m.: smear showed many damaged parasites. 3.20 p.m.: parasites diminished and many of them damaged (the morphology of the damaged parasites will be discussed later).

July 28th. 9 a.m.: no *Babesia* found; *T. annulata* and *A. marginale* present. 200 mgm. of the drug destroyed the parasites in approximately $46{,}000 \times 10^6$ infected red cells.

August 8th. *B. bigemina* appeared. They disappeared spontaneously on August 12th.

August 20th. *B. bigemina* reappeared. Both normal and abnormal forms were present. Between September 20th and January 16th, 1940, there were seven relapses, in which both normal and abnormal forms were present.

CALF NO. 27

November 8th, 1939. Animal splenectomized.

,, 14th. *B. bigemina* appeared in the blood. 8×10^6 red cells

per c.cm. were infected. Weight 45 kilo. 11 a.m. : 90 mgm. of the drug were injected. 2 p.m. : a few normal and many damaged parasites were found.

November 15th. No *B. bigemina* found; 90 mgm. of the drug destroyed the parasites in approximately $26{,}000 \times 10^6$ infected red cells. The animal was observed up to December 12th, and there were no relapses.

CALF No. 33

November 29th, 1939. The animal was examined and found to be infected with *B. bigemina*, which disappeared spontaneously by December 5th.

December 7th. Animal splenectomized.

,, 8th. *B. bigemina* reappeared in the blood.

,, 10th. Temperature 41·9° C. The animal was in distress. $6{\cdot}6 \times 10^6$ red cells per c.cm. were infected. Weight 54 kilo. 1 p.m. : received 100 mgm. of the drug. 2 p.m. : few normal and many damaged parasites were found. 3 p.m. : few parasites were found of any kind.

December 11th. 9 a.m. : blood clean. 100 mgm. of the drug destroyed the parasites in approximately $28{,}000 \times 10^6$ infected red cells within 20 hours.

CALF No. 35

December 6th, 1939. Animal splenectomized.

,, 8th. *B. bigemina* appeared ; 640,000 red cells per c.cm. were infected. Weight 50 kilo. Received 100 mgm. of the drug.

December 9th. No *B. bigemina* found.

,, 25th. *B. bigemina* (normal and abnormal forms) appeared ; they disappeared on December 27th.

January 3rd, 1940. *B. bigemina* (normal and abnormal forms) reappeared ; 7×10^6 red cells per c.cm. were infected. Weight 50 kilo. At noon 100 mgm. of the drug. were administered. 1 p.m. : very few parasites normal and abnormal found. 2 p.m. : very few abnormal forms found. 3–5 p.m. : ditto.

January 4th. 9 p.m. : no parasites found. 100 mgm. of the drug destroyed the parasites in approximately $28{,}000 \times 10^6$ infected red cells.

CALF No. 57

March 6th, 1940. Animal splenectomized.

,, 20th. Inoculated with *T. annulata* and *B. bigemina*.

,, 28th. *B. bigemina* appeared.

,, 29th. 28×10^6 red cells per c.cm. were infected. Weight 44 kilo. 10 a.m. : 100 mgm. of the drug were administered. 11 a.m. : residues of parasites found in red cells ; very few normal parasites. 1 p.m. : ditto. 2 p.m. and 3 p.m. ; only residues of parasites found in the red cells.

March 30th. 9 a.m. : no *Babesia* found.

April 8th. The animal died of *Theileria*. 100 mgm. of the drug destroyed the parasites in approximately $95{,}000 \times 10^6$ infected red cells.

BABESIA BIGEMINA IN UNTREATED SPLENECTOMIZED CALVES

CALF NO. 10

July 11th, 1939. Animal inoculated with 15 c.cm. blood from calf no. 5 (before calf no. 5 had been treated and before abnormal forms of *B. bigemina* had appeared).

July 16th–27th. *B. bigemina* found in smears. Only normal forms were present.

July 20th. Animal splenectomized (during the infection).

,, 25th. *A. marginale* appeared.

August 16th–28th. *B. bigemina* constantly present.

September 1st. The animal died of anaemia. No abnormal forms were ever found in this animal.

CALF NO. 13

July 7th, 1939. Animal splenectomized.

,, 16th. *B. bigemina* appeared in the blood.

,, 20th. Blood negative.

,, 27th. Received 70 c.cm. blood from calf no. 5 (before abnormal forms of *B. bigemina* had appeared in calf no. 5).

August 8th–November 12th. The animal had five relapses of *B. bigemina*, and died on November 12th. The blood was swarming with *Babesia*. Only normal forms were found.

CALF NO. 14

July 7th, 1939. Animal splenectomized.

,, 19th. Inoculated with *T. annulata*.

August 6th. *T. annulata* found in the blood.

,, 21st. Inoculated with 100 c.cm. blood from calf no. 5 (when normal and abnormal forms of *B. bigemina* were present).

August 28th. *B. bigemina* (normal forms) appeared and were present in the blood until September 6th.

October 4th–November 8th. There were two relapses, in which both normal and abnormal forms were present. The animal died of anaemia on November 12th.

CALF NO. 21

August 3rd, 1939. Animal splenectomized.

,, 21st. Inoculated with 200 c.cm. blood from calf no. 5 (when normal and abnormal forms of *B. bigemina* were present).

August 28th. *B. bigemina* (normal forms) appeared in the blood; they disappeared on August 31st.

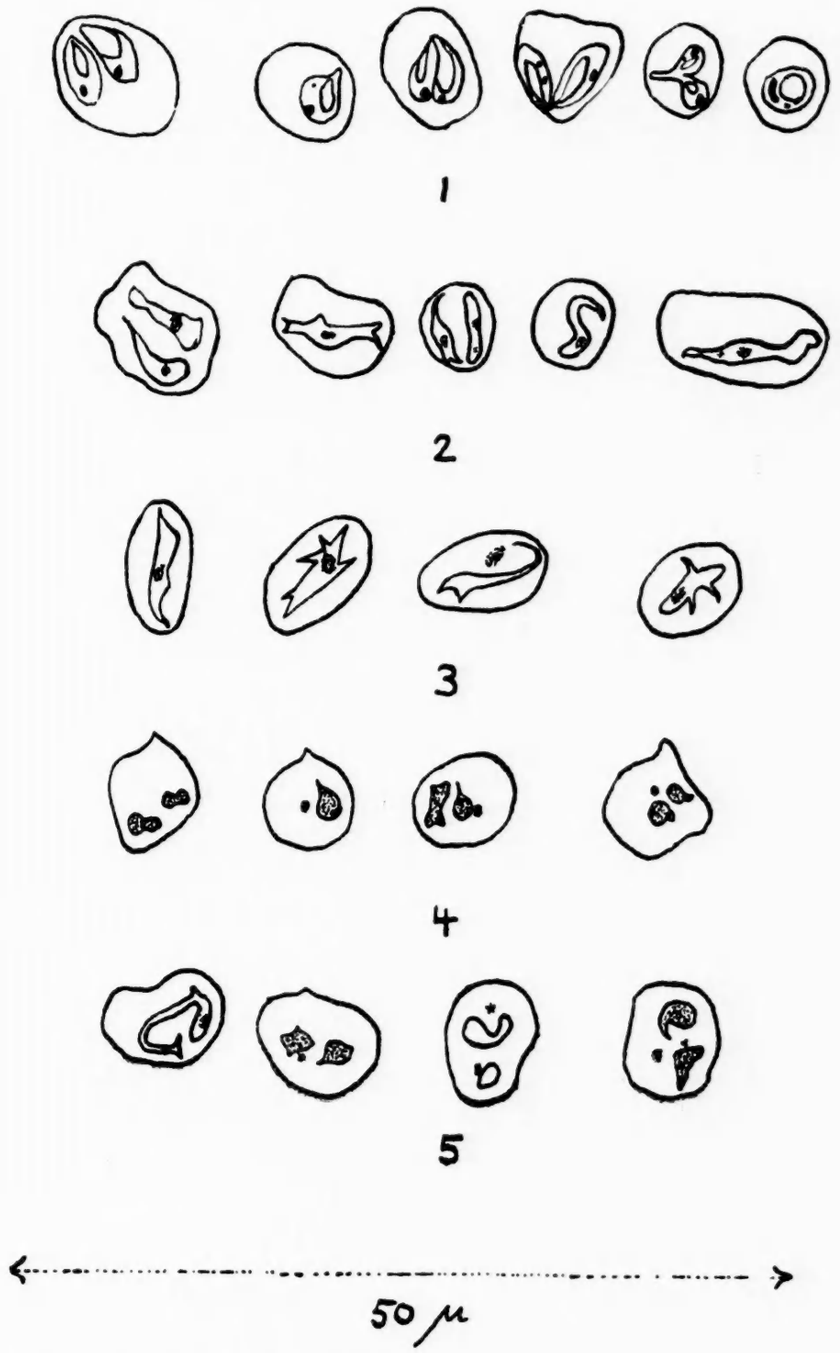

FIG. 1. Normal forms of *Babesia bigemina*.

FIGS. 2–3. Abnormal forms capable of multiplication produced by the action of 4 : 4′-diamidino stilbene.

FIG. 4. Parasites one and a half hours after intravenous injection of 4 : 4′-diamidino stilbene. Residues of parasites are seen, some without a nucleus, others with an extruded nucleus.

FIG. 5. Parasites two and a half and three hours after treatment.

September 24th–October 30th. There were six untreated relapses of *B. bigemina* with both normal and abnormal forms. On October 30th the animal died of anaemia and cachexia.

It is thus clear that animals inoculated from calf no. 5 before it contained abnormal forms of *B. bigemina* developed only normal forms of the parasite, while those inoculated with blood containing both normal and abnormal forms developed infections containing both types. It is therefore evident that the abnormality was caused by the action of the drug on the parasites.

Although the infection also disappeared irregularly in untreated splenectomized calves, it is reasonably certain that in the six treated animals the parasites were destroyed by the drug, since they invariably disappeared from the bloodstream within 24 hours after treatment.

As seen from the above protocols, the action of the drug on the parasite is very rapid and is well in evidence one hour after intravenous injection. The parasites lose their sharp outline and vacuole, and eventually the nucleus is extruded, leaving an irregular residue of protoplasm, which disappears within 18 hours. Some parasites, though damaged, retain their nuclei and are able to multiply without regaining their normal morphology. This accounts for the presence of the above-mentioned abnormal forms, which are long, stretching across the greater part of the infected red cell, amoeboid, with irregular outline, and thinner than the normal forms (figs. 2 and 3). This phenomenon was observed in two treated calves (no. 5 and no. 35), and in calves infected from treated ones (no. 14 and no. 21). We have never observed it in other cases.

Unlike Lourie and Yorke (1939) in the case of *B. canis* in puppies, we did not obtain a therapia sterilisans even when treating mild infections, e.g., calf no. 35. This is a distinct advantage in endemic centres, because acquired immunity in non-splenectomized cattle against *B. bigemina* depends on a residual infection.

SUMMARY AND CONCLUSION

1. 4 : 4′-diamidino stilbene has no therapeutic action on *Theileria annulata*, *Anaplasma marginale* and *Anaplasma ovis* in doses up to 10 mgm. per kilo. body weight.

2. The drug in doses from 2 mgm. to 4 mgm. per kilo. is effective in treating infections of *Babesiella ovis* in goats and *Babesia bigemina* in calves. In the case of *B. bigemina* the drug does not cause complete eradication of the parasites. This is a great advantage in endemic areas.

3. The action of the drug on *B. bigemina* is very rapid.

4. Morphologically abnormal forms of *B. bigemina* capable of multiplication were produced by the action of the drug in two cases.

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Lourie, E. M., and Yorke, W. (1939). Studies in chemotherapy. XXII : The action of certain aromatic diamidines on *Babesia canis* infections of puppies. *Ann. Trop. Med. & Parasitol.*, **33**, 305.

A NOTE ON THE METABOLISM OF TISSUES INFECTED WITH *LEISHMANIA DONOVANI, L. INFANTUM* AND *L. TROPICA*

BY

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(*Received for publication September 14th*, 1940)

It was shown by Adler and Ashbel (1934) that the flagellate stage of all the human Leishmanias under both aerobic and anaerobic conditions produces a considerable amount of glycolysis: for example, a study of the gas-exchange of two strains of *Leishmania donovani* gave the following results:

TABLE I

Oxygen consumption of 50×10^6 flagellates in Ringer* and bicarbonate in mm.^{3} O_2 per hour	Aerobic glycolysis of 50×10^6 flagellates in Ringer†, bicarbonate and glucose in mm.^{3} CO_2 per hour	Anaerobic glycolysis of 50×10^6 flagellates in Ringer†, bicarbonate and glucose in mm.^{3} CO_2 per hour
Strain I 23·4	7·6	19·8
Strain II 20·9	9·6	28·7

*2 c.cm. Ringer + 0·06 c.cm. of a 0·2 per cent. solution of $NaHCO_3$.
†2 c.cm. Ringer + 0·1 c.cm. of a 5·6 per cent. solution of glucose + 0·4 c.cm. of a 1·3 per cent. solution of $NaHCO_3$.

A study was undertaken of the metabolism of livers and spleens of spermophils and Syrian hamsters infected with *L. donovani* and *L. infantum*, and of spleens of Syrian hamsters infected with *L. tropica*.

METHODS

Syrian hamsters, *Cricetus auratus*, and spermophils, *Citillus citillus*, were infected with *L. donovani* and *L. infantum* by inoculating rich cultures intraperitoneally. In the case of *L. tropica* we inoculated cultures directly through a fine needle immediately under the capsule in the longitudinal axis of the spleen of Syrian hamsters. By this method a visceral infection is established in most animals within two months, and we have noted in one instance a heavy uniform infection of the skin without obvious external lesions.

At various intervals animals were sacrificed, and the gas-exchange of slices of liver and spleen were measured in a Barcroft-Warburg apparatus, according to the method of Warburg.

RESULTS

Some typical results presented in mm.^{3} O_2 per hour for oxygen consumption, and mm.^{3} CO_2 per hour for glycolysis, per 1 mgm. nitrogen (determined by micro-Kjeldahl) of the tissues used are shown in Table II. All of 33 experiments pointed uniformly in the same direction.

TABLE II

Oxygen consumption in Ringer and bicarbonate in mm.^{3} O_2 per hour per mgm. N.	Aerobic glycolysis in Ringer, bicarbonate and glucose in mm.^{3} CO_2 per hour per mgm. N.	Anaerobic glycolysis in Ringer, bicarbonate and glucose in mm.^{3} CO_2 per hour per mgm. N.	Remarks
13·4	0·5	19·2	Spleen of normal hamster
17	0	12	,, ,, ,,
7	0	18·5	,, ,, ,,
21	1·4	3·5	Liver of normal hamster
25·3	0	7·7	,, ,, ,,
39	0	19	Spleen of hamster 97 : slight infection *L. donovani*
21	28·5	27·6	Spleen of hamster 91 : heavy infection *L. donovani*
	6·6	44·6	Spleen of hamster 93 : moderate infection *L. donovani*
25·7	26·8	49·3	Spleen of hamster 320 : heavy infection *L. infantum* Malta
41·7	27·3	34	Spleen of hamster 123 : heavy infection *L. infantum* Catania
28·9	5·7	19·4	Liver of hamster 191 : heavy infection *L. infantum* Catania
26·4	3·5	21·2	Liver of hamster 270 : heavy infection *L. donovani*
31·4	26·3	54	Spleen of spermophil 3 : heavy infection *L. infantum* Malta
14	28	25	Spleen of spermophil 4 : heavy infection *L. infantum* Malta
13	30	36	Spleen of spermophil 18 : heavy infection *L. infantum* Malta
18·2	8·1	39·4	Liver of spermophil 2 : heavy infection *L. infantum* Malta
18·3	5·7	21·6	Liver of spermophil 4 : heavy infection *L. infantum* Malta
9·2	8·3	11	Spleen of hamster infected with *L. tropica*
7	7	8·5	,, ,, ,, ,, ,,
5	3	7·5	,, ,, ,, ,, ,,

Fluids as in Table I

There is no quantitative constancy in the results obtained, mainly because there were considerable variations in the intensity of infections among different

animals ; but it is clear that livers and spleens of infected animals differ from normal ones in their increased oxygen consumption, and particularly in their capacity for aerobic glycolysis. A histological examination of the tissues showed very definitely that the greater the infection in any liver or spleen, the greater is the glycolysis, particularly the aerobic glycolysis. A similar finding was made by Ashbel (1935) in the case of lymphatic glands of calves infected with *Theileria annulata.*

In the hamsters the histological picture was complex and not uniform ; apart from the infected cells there were a varying number of non-infected cells, including megakaryocytes, lymphocytes and plasma cells, and, although the parallelism between aerobic glycolysis and intensity of infection indicated that the infected cells were mainly responsible for the change in tissue-metabolism, it was not possible to exclude the effect of other cell-aggregates acting under pathological conditions. An examination of the spleens of heavily infected spermophils showed that the follicles had completely disappeared, and, except for a few occasional plasma cells, the organ consisted of a homogeneous tissue of cells, of which almost every individual was infected with Leishman-Donovan bodies. These spleens showed very high aerobic glycolysis, and we therefore conclude that the individual infected cell, in contrast to the normal reticulum cell, performs aerobic glycolysis. Sections of liver of heavily infected spermophils showed the parenchyma to be in an advanced stage of fatty degeneration, the Kupffer cells all infected, and heavy infiltration of infected macrophages both inside and outside the portal spaces. These livers also showed a marked aerobic glycolysis. The aerobic glycolysis in infected livers both in spermophils and in hamsters was less than in the spleens, because they contained relatively fewer infected cells.

EXPERIMENTS WITH LEISHMAN-DONOVAN BODIES OF *L. DONOVANI* AND *L. INFANTUM*

Leishman-Donovan bodies were liberated from the tissues by the following method. Heavily infected livers or spleens are macerated with Ringer, and the resulting pulp is slowly centrifuged for 3 minutes. The supernatant fluid contains numerous Leishman-Donovan bodies and a few blood-cells and fragments of tissue-cells. The supernatant fluid is removed and placed on ice, while the deposit is again treated with Ringer in a mortar and slowly centrifuged for 3 minutes. This process is repeated till sufficient Leishman-Donovan bodies are collected in the supernatant fluid of various centrifugations. The tubes containing the separated fluid are then slowly centrifuged for 5 minutes and the supernatant fluid is again separated. The deposit contains fragments of tissue-cells and blood-cells and numerous Leishman-Donovan bodies, and the supernatant fluid contains practically only Leishman-Donovan bodies.

The fluid from the last centrifugation is centrifuged at high speed for 10 minutes and the supernatant fluid is discarded. The deposit is collected and

washed three times in Ringer, and is finally made into a suspension in 8 c.cm. Ringer ; 2 c.cm. are used for a micro-Kjeldahl (it is difficult to count Leishman-Donovan bodies in the suspension) and the remainder is divided into three equal portions for determining oxygen consumption, aerobic glycolysis and anaerobic glycolysis in a Barcroft-Warburg apparatus.

Although the majority of Leishman-Donovan bodies are lost, the above process yields a pure suspension of Leishman-Donovan bodies, and from a heavily infected animal sufficient parasites for our purpose can be obtained.

In our first experiments, traces of tissue were still present in the suspension and relatively small amounts of aerobic glycolysis were observed ; but when meticulous care was taken to prepare pure suspensions of Leishman-Donovan bodies no appreciable amounts of aerobic glycolysis could be determined.

In this respect the Leishman-Donovan bodies differ fundamentally from the flagellate stage. It should be pointed out, however, that the manipulations and frequent washings necessary to obtain a clean suspension of Leishman-Donovan bodies took up to 5 hours, and the parasites may have been damaged during the process, although they stained normally and oxygen consumption and anaerobic glycolysis were easily demonstrated.

SUMMARY

1. The metabolism of slices of livers and spleens of Syrian hamsters and spermophils infected with *Leishmania donovani* and *L. infantum*, of spleens of Syrian hamsters infected with *L. tropica*, and of suspensions of Leishman-Donovan bodies of *L. infantum* and *L. donovani*, was examined in a Barcroft-Warburg apparatus.

2. Infected tissue (liver and spleen) produces aerobic glycolysis, and in this respect it differs from normal tissue.

3. The more intense the infection, the greater is the glycolysis, both aerobic and anaerobic—particularly the former. Oxygen consumption is also increased.

4. The individual infected cell produces aerobic glycolysis.

5. The Leishman-Donovan body freed from tissue produces no appreciable aerobic glycolysis under the conditions of our experiments, and in this it differs fundamentally from the flagellate stage.

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ONCHOCERCIASIS IN TANGANYIKA TERRITORY

BY

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(*Received for publication September 26th*, 1940)

The purpose of this paper is to describe the distribution of onchocerciasis in the south-west portion of Tanganyika Territory, East Africa.

LITERATURE

Fischer (1932; also private communication) observed nodules containing *O. volvulus* in natives living near Lake Nyasa at Itete and Matema, and in natives from the Ulanga region attending the mission hospitals of Lupembe and Ilembula. The nodules were as big as a cherry or slightly larger, and were situated on the chest-wall, abdomen, hips, leg, neck and forehead. No eye symptoms of onchocerciasis were seen. Cases with similar local lesions were later observed by Dr. Eckart at Itete; but (according to Fischer) Fülleborn, who investigated the district near Lake Nyasa in 1900, had not seen onchocercal nodules. Simuliidae have been collected at Karonga, Nyasaland, by Dr. W. Hood-Dye (private communication). Dr. J. Harkness (private communication) about the same period discovered *Simulium* occurring at Njombe, and specimens collected there by Dr. R. R. Scott in November, 1938, were identified as *S. damnosum* and *S. neavei* by Mr. E. G. Gibbins. In 1938 Dr. A. V. Clemmy removed a tumour, which proved to contain onchocerca, from the scalp of a girl aged 13 years, belonging to Katumba, a village four miles north-west of Tukuyu. It may also be noted that *S. hirsutum* occurs at Morogoro, and *S. vorax* at Amani (Pomeroy, 1922); but there is no record of transmission of *Onchocerca* by these last-named species.

PRESENT INVESTIGATIONS

The investigations here described were made during May, 1939, at the end of the rainy season. In each place visited, as many natives as possible were examined, e.g. hospital patients, prisoners, labourers, or school-children. A quick palpation was made of the body down to the waist, and the eyes were examined. In all cases where there was any suggestion of nodules or of eye

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lesions, as well as in many other cases besides, a thin shaving of skin was taken from between the shoulders or near the supposed nodule. The skin-shaving was placed in saline on a slide and teased out; after standing for a quarter of an hour it was examined under the microscope.

RESULTS

The results of these investigations are shown in Table I. The natives are

TABLE I

Showing the result of examining natives from various places for infestation with *Onchocerca volvulus*

Origin of natives	No. examined		No. positive (microfilariae)
	By palpation	Microscopically	
Chunya	11	8	0
Mbeya	70	24	0
Sumbawanga	3	2	0
Tukuyu	165	39	3
Ilembula	211	13	0
Njombe	102	49	4
Uwemba	45	7	0
Malangali	34	26	0
Mufindi	3	3	1
Iringa	143	43	0
Dodoma	2	2	0
Bukoba	20	9	0
Ufipa*	202	0	0
Songea	10	4	0
Rhodesia	31	3	0
Nyasaland	27	4	0
Portuguese East Africa	8	0	0
Totals	1,087	236	8

* Examined at Kigoma

listed under their place of origin. The actual places visited were Chunya, Mbeya, Tukuyu, Ilembula, Njombe, Uwemba, Malangali, Iringa, Bukoba and Kigoma.

Details of the eight cases infested with *Onchocerca volvulus* are as follows:

1. Man, 35 years old, from Itete Mission, Tukuyu. Small nodule on ribs; this was removed at operation and shown to contain adult onchocercae. Eyes normal.

2. Man, 25 years old, from Masoko, Tukuyu. Small pellet in skin on back. Eyes normal.

3. Man, 25 years old, from Itete Mission, Tukuyu. No nodules. One eye had just undergone needling for cataract. Other eye normal. (Seen at Dodoma.)

4. Woman, 55 years old, from Idunda, Njombe. Nodule, 1–2 mm., between scapulae. Corneal ulcer over most of one eye ; duration two years.

5. Man, 35 years old, from Igomini, 50 miles south of Njombe. No nodules. Eyes normal.

6. Woman, 25 years old, from Lupembe Mission, Njombe. No nodules. Eyes normal.

7. Man, 18 years old, from Utengule, Njombe. No nodules. Opacities on both sides of corneae.

8. Man, 20 years old, from Mufindi. Diffuse thickening of subcutaneous tissues over first dorsal spine. Eyes normal.

It is seen that in only one case was a definite nodule present. Eye lesions were present in three out of the eight cases, but it was impossible to determine whether they were due to *Onchocerca* or to other causes.

METEOROLOGY AND ENTOMOLOGY

The altitude and rainfall of the various places concerned are shown in Table II. Their geographical positions are shown in the map given in a previous paper (Hawking, 1940).

TABLE II

	Altitude	Annual rainfall
Chunya ...	4,900 feet	31·9 inches
Mbeya ...	6,000 „	35·6 „
Tukuyu ...	5,300 „	101·4 „
Ilembula ...	4,560 „	23·4 „
Njombe ...	6,400 „	42·6 „
Uwemba ...	? 7,000 „	—
Malangali ...	4,700 „	30·9 „
Mufindi ...	6,102 „	39·7 „
Iringa ...	5,578 „	26·3 „
Bukoba ...	3,740 „	76·2 „

The temperatures are mostly low for the tropics, but exact figures are not available. Some of the localities merit further description.

Chunya and Mbeya lie among hilly country, but no *Simulium* could be discovered in short visits to streams at Mbeya or to the Lupa River near Lupa Market.

Tukuya. This region has a heavy rainfall and numerous mountain-streams which would appear suitable for *Simulium.* It was not possible to make a search, but no reports of small black biting flies could be obtained from Europeans

resident in the district. Apparently *Simulium* has not yet been identified in this region, although presumably it must be present. The natives go almost completely naked, and so would be widely exposed.

Ilembula lies in a flat plain. There is a small turbid stream nearby, but no *Simulium* could be discovered and the stream appeared unsuitable for breeding. Presumably the cases, observed here by Fischer, came from a distance.

Njombe. Immediately next to the station there is a waterfall about 200–300 feet high. At the bottom of the fall (4 p.m.) the flies were very numerous and voracious, and constituted a severe pest (native name, Maswámu). They attacked the legs especially, and seemed to be more attracted to a dark surface than a light one. No larvae could be found on the stones or weeds in this part of the stream, although the water must be exceptionally aerated. Natives rarely visit the foot of the fall. At the head of the fall only occasional flies were noted, but the natives use the flat rocks for washing clothes, and infection could probably easily occur. Here larvae and pupae were found in great numbers, though the kind assistance of Mr. G. H. Swynnerton. They were attached to coarse green plants submerged in the current between the rocks. At this level, although the flow is rapid, the surface of the water is still smooth and unbroken, and there is no reason to suppose exceptional aeration, since the stream for more than a mile above this point is smooth and not particularly swift. Specimens of larvae, pupae and adults were sent to Mr. E. G. Gibbins at Entebbe, who kindly reported as follows :

' Of the adults taken while biting, 81 were *S. damnosum* and 5 *S. neavei.* Among the pupae were examples of *S. damnosum*, *S. medusaeforme* and *S. lepidum.* Many of the larvae were too young to establish their identity, but I was able to recognize the larvae of *S. damnosum* among them.'

Uwemba Mission lies about 20 miles south of Njombe. A waterfall was visited (9.0 a.m.) between Njombe and Uwemba, and two others beyond the Mission (4 p.m.), but no *Simulium* could be discovered. The monks of the Mission reported that small black biting flies occurred at Kifanye and on the Mauli River towards Songea. Rumours of similar flies were received at Malangali and Iringa (the Little Ruaha River), but none could be discovered there or at Sao Hill.

DISCUSSION

It seems clear that onchocerciasis does occur in the districts around Tukuyu and Njombe, and probably also in the Mufindi neighbourhood ; but the number of persons affected is small, and from a public health point of view the infestation is relatively unimportant. The number of infested persons seen was too small to determine whether or not the disease is responsible for eye lesions.

Serum from one of the above cases of onchocerciasis was sent by air-mail to Dr. N. Hamilton Fairley to test for complement deviation against Dirofilarial

antigen ; he kindly reported that no deviation occurred. During this work the eyes of large numbers of natives were examined, and the frequency of lesions, unassociated with *Onchocerca*, may be of interest from the point of view of public health. Out of 690 persons, in whom no evidence of onchocerciasis was found during the above examinations, 5 had acute conjunctivitis, 29 had corneal opacities, 3 had cataract, 7 were blind in one or both eyes, and in 7 there were scars of the iris distorting the pupil, i.e., about 7 per cent. had eye lesions of one type or another.

SUMMARY

1. A search for onchocerciasis in the south-western portion of Tanganyika Territory revealed a few cases in natives from the districts around Tukuyu, Njombe and Mufindi. The number of persons infested is relatively small, and the condition is probably unimportant at the present time. Natives from Bukoba and Ufipa were negative on examination.

2. A description is given of a breeding-place of *Simulium damnosum*, *S. neavei*, *S. medusaeforme* and *S. lepidum* which occurs at Njombe.

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A SHORT REPORT ON THE USE OF 4:4′-DIAMIDINO STILBENE IN THE TREATMENT OF HUMAN SLEEPING SICKNESS

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This paper records the clinical work and the laboratory findings in cases of trypanosomiasis in the Gambia who were treated with 4 : 4′-diamidino stilbene (see Lourie and Yorke, 1939 ; Yorke, 1940). The investigations were carried out at the request of Professor Warrington Yorke.

METHODS

The drug, which was received in ampoules of 50 and 100 mgm., is a white powder slowly soluble in water. As a rule the drug was given intravenously, but a few cases were treated by the intramuscular route ; the data received about the intramuscular method of treatment were, however, inadequate, and they have therefore been omitted from the present findings. In cases treated by intravenous injection the drug was given twice weekly in doses of 1 mgm. per kilo. body weight. The first set of cases were treated in hospital and were kept under very close observation ; later cases were treated as out-patients in country districts. It will be seen from the table (pp. 220-1) that some cases received seven injections, others ten injections. This was due to the fact that originally two series of tests were made, which were carried out at different times.

When administered by the intravenous route, the drug is somewhat upsetting to the patients for the first two or three injections. Headache, sweating, tachycardia, vomiting or retching may occur, and there may be weakness of the pulse, associated with a marked fall in blood-pressure. The symptoms are of short duration and last only a few minutes, but they may be rather alarming at the time. Some of the patients fainted, but they revived quickly. After the first three injections, however, only transient headache occurs. If the patient is cold before being treated, the symptoms are much more likely to be severe. The drug should be prepared fresh before use, as material made up for more than six hours appears to be more toxic than that freshly prepared. The reason for this increase in toxicity is not yet clear.

The following routine was carried out in all cases. After clinical diagnosis, blood examinations were made, which were followed by gland puncture and lumbar puncture. Then, if these examinations showed the case to be positive (and only proved positive cases were used in the investigation), treatment was

begun. As stated above, injections of 1 mgm. per kilo. body weight were given twice weekly. Before the second injection, blood examination and gland puncture were repeated, and if either was found to be positive the examinations were again repeated before the third injection, and so on until negative results were obtained. When the course of treatment had been completed another lumbar puncture was performed, and whenever possible a final lumbar puncture was carried out one year after the course of treatment. Although in a large number of cases this final examination could not be carried out, clinical reports were received of almost all cases.

After a course of 4 : 4′-diamidino stilbene had been commenced, tryparsamide was given only where an obvious clinical relapse occurred. But, since sleeping sickness varies considerably in virulence in different parts of Africa, a small series of cases were treated with tryparsamide only (two injections of 2 gm., followed by 3 gm. weekly), in order to ascertain how cases in the same district respond to the arsenical drug, and to give some idea of the virulence of the disease and the type of case in that district. For the same reason, a small series of cases were treated with Bayer 205, of which 1 gm.* was administered twice weekly, irrespective of body weight. In these cases the urine was tested regularly : slight albuminuria was found after the first four injections, but during the second half of the course of treatment the urine became clear.

RESULTS

In order to facilitate comparison, the cases have been grouped into series and set together in one table (see pp. 220-1). The duration of the disease (so far as could be ascertained) is indicated by the fraction in column 2 (e.g., 3/12 means three-twelfths of a year, or three months). This, however, is probably in most cases less than it should be, for the history given by patients is frequently very inaccurate, and complaint is only made when signs and symptoms become very marked.

The cases have been arranged into six groups : (*a*) Those treated with 4 : 4′-diamidino stilbene, with lumbar puncture performed before and after treatment, and after an interval of, as nearly as possible, one full year after the completion of treatment. (*b*) Those treated with 4 : 4′-diamidino stilbene, with lumbar puncture performed before and after treatment, but not a year later, either because the patient could not be traced or because he refused further investigation (this was exceptional, as most patients were very helpful and willing to co-operate). (*c*) Those which died before the course of treatment with 4 : 4′-diamidino stilbene was completed. (*d*) Those which relapsed or did badly after the course of 4 : 4′-diamidino stilbene and were subsequently given a course of tryparsamide. (*e*) Controls treated with tryparsamide alone ; in

* This dose is much larger than that recommended by the makers, and is, in my opinion, unwise ; it was not given with my advice.

none of these cases was lumbar puncture performed a year after the completion of treatment, because in all cases the cerebrospinal fluid was very nearly, if not quite, normal at the end of treatment. (*f*) Controls treated with Bayer 205 alone ; in these cases lumbar puncture was performed before and after treatment, but not a year later ; the cases in this group were not seen personally by the present writer at the end of the year's interval, as unforeseen circumstances prevented the necessary visit to the station where they were treated, but they were all reported to be well.

All cases in which lumbar puncture was performed (except those marked ' Reported well ' or ' Unknown ') were seen personally by the writer. Those marked ' Very well ' and ' Excellent ' were seen, but for various reasons lumbar puncture was not carried out. It will be observed that in three cases there was an increase in the cerebrospinal fluid protein following treatment with Bayer 205. The fact that no lumbar puncture was performed after an interval of a year makes it impossible to form an opinion whether this indicates that the disease was still active, or whether it was due only to a temporary alteration caused by the drug.

Estimations of the protein in the cerebrospinal fluid were made by means of Ravaux's albuminodosometer, using sulphosalicylic acid to precipitate the protein. The cell counts refer to the number of cells per c.mm. In all cases where the blood was found to contain trypanosomes before the commencement of treatment it became negative within two days of the first injection of 4 : 4′-diamidino stilbene. This was also found to be true in cases treated with Bayer 205. Cervical glands in some cases became negative after the first injection of 4 : 4′-diamidino stilbene, and in most cases within two days of receiving the second injection ; in only one case (no. 28) were trypanosomes found after the third injection. Diamidino stilbene and Bayer 205 appear to be equally effective in clearing the blood-stream and the cervical glands of trypanosomes.

A case not included in the present series is of some interest : a child aged five months, whose blood was found to contain malaria parasites and trypanosomes, was given 5 mgm. of 4 : 4′-diamidino stilbene in solution by injection into the superior sagittal sinus via the anterior fontanelle. No apparent reaction was noted, and the clinical condition of the child showed very marked improvement during the next three days. But the parents then removed the child, against medical advice, and no further observations were possible.

OBSERVATIONS

The object of this short paper on the use of 4 : 4′-diamidino stilbene is to correlate in a concise manner the data collected concerning its use in treatment of cases of sleeping sickness in the Gambia. No prolonged discussion is attempted, and a few observations only will be made.

Table showing the results of treating cases of sleeping sickness

Case no.	Duration of disease	Signs	Condition	Diagnosis	Lumbar puncture before treatment		Treatment : no. of doses
					Cells	Protein	
			(*a*) Cases treated with 4 : 4′-diamidino stilbene ; lumbar puncture performed before				
1	3/12	H.N.W.	Good	G.	2	0·025%	10
3	3/12	H.S.N.	Very good	G.	2	0·025%	10
13	6/12	H.S.N.D.	Good	G, B.	5	0·02 %	10
14	9/12	N.W.	Poor	G.	5	0·03 %	10
15	2/12	H.N.D.	Good	G.	8	0·05 %	10
16	3/12	H.N.	Excellent	G.	8	0·03 %	10
17	1/12	H.N.	Excellent	G.	8	0·05 %	10
19	2/12	H.M.N.	Good	G,B.	10	0·055%	10
20	9/12	H.N.D.	Fair	G.	10	0·03 %	7
27	2/12	H.N.	Excellent	G.	114	0·035%	10
			(*b*) Cases treated with 4 : 4′-diamidino stilbene ;				
2	48/12	M.N.H.	Fair	G.	2	0·04 %	10
4	8/12	H.N.	Excellent	G.	2	0·02 %	7
5	3/12	H.N.	Excellent	G.	3	0·02 %	7
6	6/12	H.N.M.P.	Fair	G.	3	0·03 %	10
7	6/12	N.D.	Good	G.	3	0·03 %	10
8	1/12	H.N.W.	Good	G,B.	3	0·02 %	10
9	3/12	N.	Excellent	G.	5	0·03 %	7
11	6/12	M.N.	Fair	G.	5	0·04 %	7
12	24/12	H.N.M.W.D.	Fair	G,B.	5	0·035%	10
22	1/12	H.N.	Good	G,B.	20	0·03 %	10
23	12/12	H.M.N.	Fair	G,B.	20	0·05 %	10
24	12/12	H.N.	Poor	G.	45	0·045%	10
25	2/12	H.N.M.W.	Fair	G.	62	0·09 %	7
30	3/12	H.N.D.W.S.	Bad	G,B.	176	0·055%	10
33	1/12	H.N.	Excellent	G.	400	0·045%	10
34	4/12	H.N.S.	Good	G.	540	0·045%	10
			(*c*) Cases known to have died during				
18	6/12	H.M.N.E.	Fair	G.	10	0·03 %	10
31	1/12	H.N.W.D.E.	Poor	G.	275	0·06 %	1
32	3/12	H.N.S.E.	Fair	G.	350	0·2 %	3
35	3/12	H.S.M.N.	Poor	G,B.	610	0·5 %	7
			(*d*) Cases which relapsed or did badly after the course of 4 : 4′-				
26	6/12	H.G.S.	Poor	G.	107	0·04 %	2
28	12/12	H.N.D.W.	Very poor	G.	128	0·08 %	10
29	6/12	H.N.M.	Fair	G,B,F.	150	0·12 %	10
36	10/12	H.S.N.D.	Fair	G,B.	950	0·1 %	7
							(*e*) Cases treated with
37	6/12	H.N.	Good	G,B.	3	0·025%	10
39	12/12	S.N.H.M.	Fair	G,B.	5	0·04 %	10
42	48/12	N.H.	Good	G,B.	18	0·03 %	10
43	6/12	N.H.	Good	G,B.	108	0·045%	10
44	24/12	H.W.N.	Good	G.	142	0·04 %	10
45	3/12	N.H.	Poor	G.	360	0·05 %	10
46	6/12	H.N.S.	Bad	G,F.	510	0·15 %	10
							(*f*) Cases treated
48	6/12	H.M.N.	Good	G,B.	2	0·04 %	10
49	6/12	H.N.P.W.	Fair	G.	3	0·04 %	10
50	24/12	N.	Good	G,B.	5	0·03 %	10
51	6/12	H.N.D.	Good	G,B.	6	0·03 %	10
52	6/12	H.M.N.D.	Fair	G.	7	0·05 %	10

EXPLANATION

B.—trypanosomes found in blood-specimens.
D.—dermatitis, dry skin with itching.
E.—some excitability in patient's manner.
F.—trypanosomes found in cerebrospinal fluid.
G.—trypanosomes found in lymph glands.
H.—headaches complained of on questioning.

with 4 : 4′-diamidino stilbene, with tryparsamide, and with Bayer 205

Clinical result	Lumbar puncture after treatment		Progress	Further treatment	Result	Lumbar puncture after one year		Clinical state
	Cells	Protein				Cells	Protein	
and immediately after treatment, and one year after the completion of treatment								
Good ; signs gone	1	0·02 %	Good	None		2	0·02 %	Cured
Excellent	2	0·025%	Good	,,		2	0·02 %	Cured
Excellent	2	0·025%	Good	,,		25	0·02 %	Cured
Improved	2	0·03 %	Good	,,		5	0·02 %	Cured
Good	88	0·06 %	Doubtful	,,		250	0·04 %	Doubtful
Excellent	8	0·03 %	Good	,,		8	0·02 %	Cured
Excellent	4	0·04 %	Good	,,		2	0·025%	Cured
Improved	15	0·045%	Good	,,		4	0·02 %	Cured
Good ; appears cured	30	0·02 %	Good	,,		88	0·02 %	Doubtful
Excellent	2	0·02 %	Good	,,		2	0·02 %	Cured
lumbar puncture performed before and after treatment								
Better	2	0·04 %	Good	None		Not performed		Reported well
Excellent	5	0·02 %	Good	,,		,,	,,	Reported well
Excellent	5	0·02 %	Good	,,		,,	,,	Very well
Improved	2	0·03 %	Good	,,		,,	,,	Reported well
Very good	3	0·04 %	Good	,,		,,	,,	Very well
Excellent	9	0·025%	Good	,,		,,	,,	Very well
Excellent	2	0·03 %	Good	,,		,,	,,	Very well
Improved	5	0·04 %	Good	,,		,,	,,	Very well
Good	9	0·025%	Good	,,		,,	,,	Very well
Very good	3	0·02 %	Good	,,		,,	,,	Very well
Improved	40	0·08 %	Unknown	,,		,,	,,	Unknown
Improved	35	0·025%	Good	,,		,,	,,	Reported well
Unsatisfactory	300	0·1 %	Unsatisfactory	,,		,,	,,	Unknown
Much better	233	0·06 %	Doubtful	,,		,,	,,	Unknown
Very satisfactory	870	0·07 %	Good	,,		,,	,,	Excellent
Unchanged	730	0·1 %	Good	,,		,,	,,	Reported well
treatment with 4 : 4′-diamidino stilbene								
Better ; excitable	400	0·07 %	Died 4 months later, with sub-acute maniacal condition.					
Died (? pneumonia) 4 days after first injection. Blood contained very heavy infection of malaria.								
Died in a collapsed state. Blood-pressure fell daily for a week until it was very low.								
Very much better. Died about 4 months after completion of treatment. Began to sleep again. Terminal pneumonia.								
diamidino stilbene and which were subsequently given a course of tryparsamide								
Improved	225	0·03 %	Worse	Tryparsamide	Cured	3rd l.p. : 10 cells ; 0·02% protein.		
Improved	130	0·045%	Relapsed	,,	,,	Not performed		
Improved	350	0·15 %	Relapsed	,,	After 3 injections went blind and left hospital.			
Improved ; relapsed	2,705	0·15 %	Worse	,,	Cured	3rd l.p. : 51 cells ; 0·02% protein.		
tryparsamide only								
Excellent	4	0·02 %	Good	None	Cured	Not performed		Cured
Excellent	2	0·02 %	Good	,,	,,	,,	,,	Cured
Excellent	5	0·02 %	Good	,,	,,	,,	,,	Cured
Very good	10	0·02 %	Good	,,	,,	,,	,,	Cured
Excellent	4	0·03 %	Good	,,	,,	,,	,,	Cured
Excellent	11	0·02 %	Good	,,	,,	,,	,,	Cured
Rapid improvement	25	0·02 %	Good	,,	,,	,,	,,	Cured
with Bayer 205 only								
Unchanged	2	0·06 %	Good	None	Cured	Not performed		Reported well
Unchanged	2	0·07 %	Good	,,	,,	,,	,,	Reported well
Unchanged	6	0·025%	Good	,,	,,	,,	,,	Reported well
Unchanged	14	0·03 %	Good	,,	,,	,,	,,	Reported well
Unsatisfactory	14	0·06 %	Good	,,	,,	,,	,,	Reported well

OF LETTERING

M.—muscular pains.
N.—neck-glands enlarged, posterior lymph chain.
P.—pyrexia noticed by the patient.
S.—sleeping in the day-time.
W.—marked loss of weight.

It is clear that the drug cures sleeping sickness in the early and intermediate stages. It is not suitable for cases in which the cerebrospinal fluid protein is above 0·05 per cent., for here an initial improvement is followed by relapse. Where the cell count is high in relation to the protein, however, the prognosis is much better. In some cases there is considerable difficulty in interpreting the laboratory findings, and the possibility of the existence of other diseases, not detected clinically, must be borne in mind (note case 33).

The series of cases treated with tryparsamide and Bayer 205 are very small, and conclusions cannot be drawn from them. The figures are merely suggestive.

SUMMARY AND CONCLUSIONS

1. 4 : 4′-diamidino stilbene is a water-soluble drug suitable for intramuscular or intravenous injection in the treatment of early cases of trypanosomiasis in human beings.

2. The drug can be given in doses up to 1 mgm. per kilo. body weight. Larger doses are not altogether safe by the intravenous route.

3. Injection of the drug causes marked stimulation of the autonomic nervous system. The disturbance is temporary and no permanent harm results. The symptoms thus caused decrease with subsequent injections.

4. There is a rapid amelioration in all symptoms of sleeping sickness, and physical signs improve quickly.

5. Cases in which the cerebrospinal fluid protein is above 0·05 per cent. are not suitable for treatment with 4 : 4′-diamidino stilbene.

6. No eye-symptoms were complained of during treatment.

7. The drug does not cause albuminuria.

8. Diamidino stilbene has an advantage over tryparsamide in the treatment of early cases of trypanosomiasis,as treatment can be carried out in half the time necessary for treatment with tryparsamide.

9. When injected intramuscularly, the drug is not irritant and is very useful in the treatment of young children, where the veins are small and difficult to enter. Intramuscular tryparsamide is too irritant for the treatment of young children.

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MISCELLANEA

A COMPARISON OF THE BIOLOGICAL ACTION OF PLASMOQUINE DIHYDROCHLORIDE (BAYER) AND PAMAQUIN DIHYDROCHLORIDE (I.C.I.)

Imperial Chemical Industries Limited have synthesized a substance—pamaquin—which they believe is chemically identical with plasmoquine (Bayer). As plasmoquine is not a crystalline substance, and as there are no chemical tests by which certain isomers can be distinguished from plasmoquine, it was considered necessary, before placing pamaquin on the market, to subject the substance to critical examination, in order to ascertain whether its biological effects are identical with those of plasmoquine. The following tests were therefore carried out at the Liverpool School of Tropical Medicine.

Toxicity. This was tested in canaries, hens, mice and monkeys, and no difference was discovered between the toxicity of the two preparations.

Plasmodicidal action. Tests were made on malaria infections in canaries (*Plasmodium relictum*), in monkeys (*P. knowlesi*), in fowls (*P. gallinaceum*) and in man (*P. falciparum* and *P. malariae*), with the following results.

Plasmodium relictum *infection in canaries*

Infection was carried out in the usual way by blood inoculation from a bird showing parasites in the peripheral circulation. Each drug was administered four hours after inoculation with infected blood, and a dose was given on each of six consecutive days.

The results summarized in Table I show that pamaquin (I.C.I.) is just as effective as plasmoquine (Bayer) in the treatment of *Plasmodium relictum* infection of canaries.

Plasmodium knowlesi *infection in* Macacus rhesus

The two monkeys were infected by blood inoculation from the same animal on the same day, and treatment was commenced six days later, when the animals were approximately equally infected. The compounds were administered intramuscularly in a dose of 3·3 mgm. per kilo. on each of four consecutive days. Details are shown in Table II.

The results there summarized indicate that pamaquin (I.C.I.) is just as effective as plasmoquine (Bayer) in the treatment of *Plasmodium knowlesi* infection of monkeys.

TABLE I

Comparing the effects of plasmoquine (Bayer) and pamaquin (I.C.I.) in *P. relictum* infection in canaries

Bird no.	Drug	Dose per 20 mgm. bird	Day of appearance of parasites in peripheral blood
95	Plasmoquine 2HCl (Bayer)	6 × 0·02 mgm.	Negative up to 40th day
96			16
97		6 × 0·04 mgm.	Negative up to 40th day
98			,, ,, ,,
99	Pamaquin 2HCl (I.C.I.)	6 × 0·02 mgm.	,, ,, ,,
100			,, ,, ,,
101		6 × 0·04 mgm.	,, ,, ,,
102			,, ,, ,,
92	Controls		5
93			7
94			5

Remarks.—The birds which remained negative up to the 40th day became infected on reinoculation, and had therefore been sterilized.

Gametocidal activity tested with Plasmodium gallinaceum *infection in fowls*

The fowls used were about 18 weeks old and were infected by mosquitoes. Figs. 1 and 2 show data relating to two fowls treated with 2·5 mgm. per kilo. of plasmoquine (Bayer) and pamaquin (I.C.I.) respectively. The curve showing the expected course of an untreated infection was constructed from data relating to five fowls, about 12 weeks old, whose infections were followed by means of parasite counts. As the fowls used in the present study were older, it is probable that the curve errs somewhat on the high side. The figures also show the times at which batches of mosquitoes were applied, and the magnitude of the subsequent average oöcyst counts per stomach.

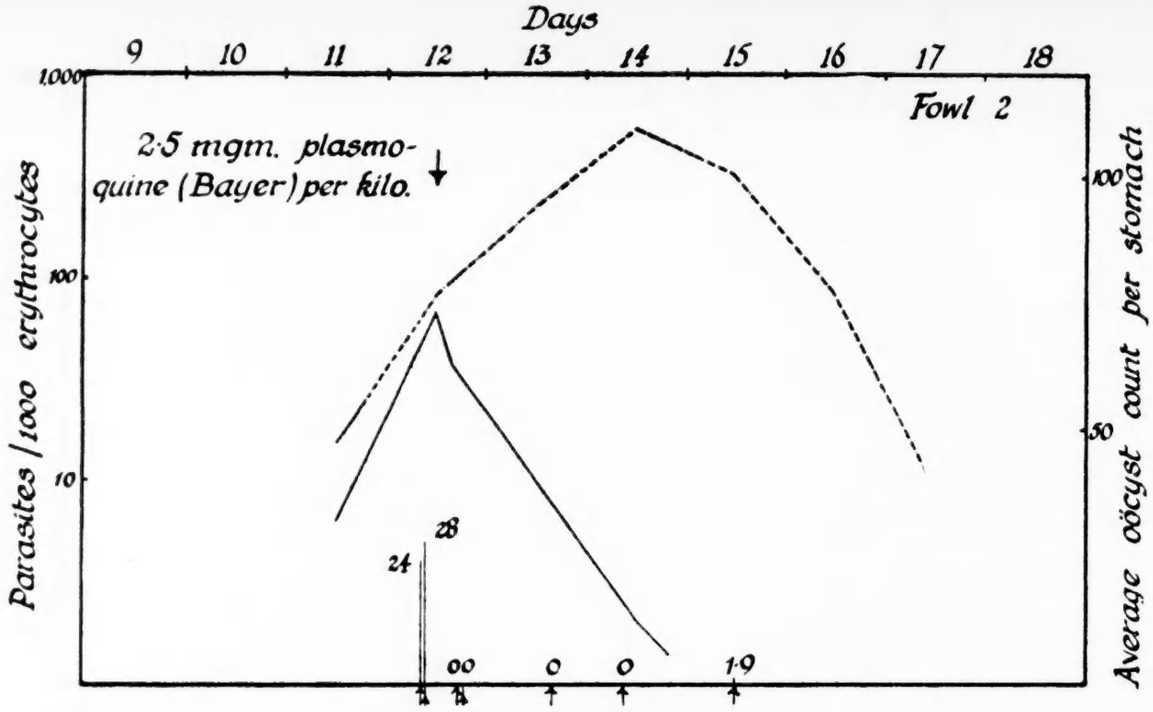
Both drugs, in doses of 2·5 mgm. per kilo., produced complete sterilization of the gametocytes in about six hours, and this persisted in both cases for at least 48 hours afterwards. The low average oöcyst count which appeared in a batch fed on the plasmoquine-treated fowl 72 hours after treatment is not considered to indicate any significant difference between the drugs. The effect, however, is seen not to be purely a gametocidal one with no effect on the general reproductive activity of the parasite, for the course of the general infection as shown by the total parasite curve is cut short to a greater degree than can be explained merely by the greater age of the fowls.

TABLE II

Comparing the effects of plasmoquine (Bayer) and pamaquin (I.C.I.) in *P. knowlesi* infection in monkeys

Date	Blood-parasites per field	Drug	Blood-parasites per field	Drug
30. 9.40	2	Plasmoquine	2	Pamaquin
1.10.40	5	"	1	"
2.10.40	53/100	"	74/100	"
3.10.40	19/100	"	10/100	"
4.10.40	7/100		0/200	
5.10.40	0/150		0/150	
6.10.40	0/200		0/200	
7.10.40	7/50		0/200	
8.10.40	10/50		0/200	
9.10.40	3–4	"	0/150	
10.10.40	9	"	6/50	
11.10.40	4	"	1	
12.10.40	15/50	"	2	"
13.10.40	0/100		4	"
14.10.40	0/100		41/50	"
15.10.40	1/200		1/200	"
16.10.40	4/100		1/100	
17.10.40	1		4/200	
18.10.40	6–7		2/100	
19.10.40	15	"	4/150	
20.10.40	8/50	"	40/50	
21.10.40	7/50	"	37/50	
22.10.40	5/100		96/50	
23.10.40	8/100		23/100	
24.10.40	6/100		2	
25.10.40	15/100		33/50	
26.10.40	2		23/50	
27.10.40	1		12/50	
28.10.40	1–2		18/50	
29.10.40	41/50		5/50	
30.10.40	3		3/100	
31.10.40	63/50		6/50	
1.11.40	63/50		4/100	
8.11.40	4/100		6/100	
	Animals remained well			

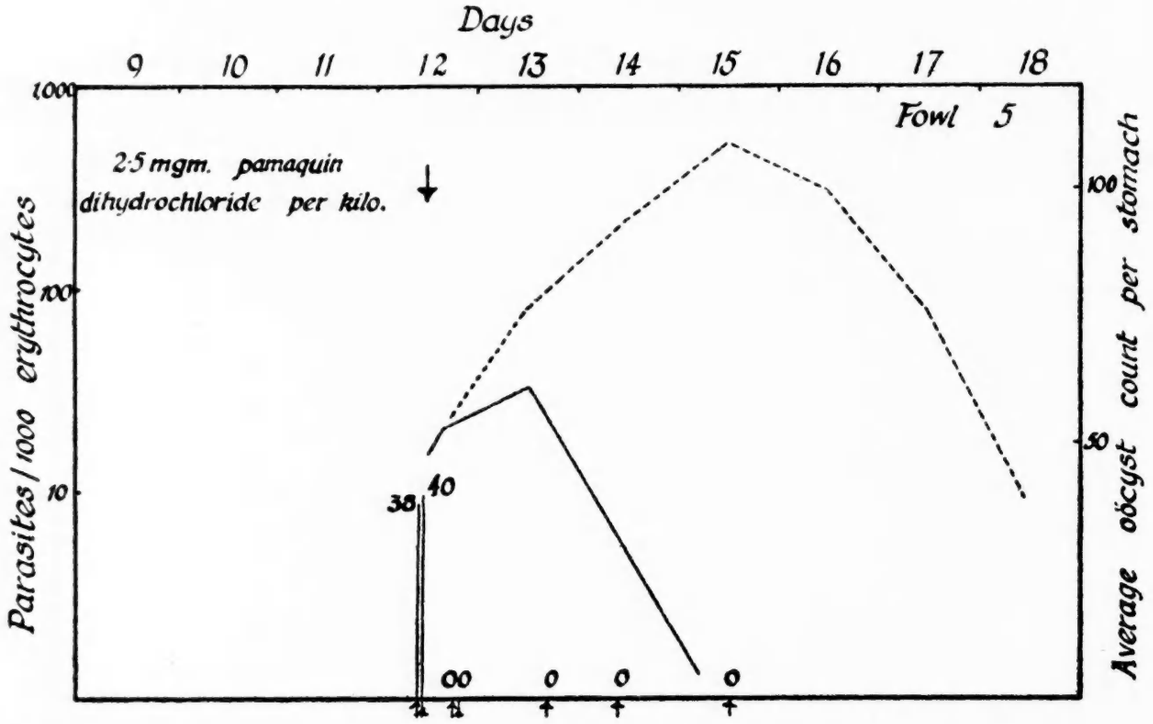
Doses of 2 mgm. and 1·5 mgm. per kilo. were also tested for each drug in the same manner. In the case of the dose of 2 mgm. per kilo., plasmoquine produced complete sterilization for five days after treatment, but pamaquin just



FOWL 2: AGE AT INOCULATION (BY MOSQUITO) ABOUT 18 WEEKS; PLASMOQUINE DOSE 2·5 MGM. PER KILO.

FIG. 1. Graphs showing total parasite count in the treated fowl and expected course of the same curve in an untreated infection (logarithmic ordinates). The lower arrows show the times of application of batches of *Aëdes aegypti*, and the corresponding vertical lines the average oöcyst count for each batch.

——— denotes parasites per 1,000 erythrocytes (experimental fowl).
- - - - „ parasites per 1,000 erythrocytes in an average untreated infection.



FOWL 5: AGE AT INOCULATION (BY MOSQUITO) ABOUT 18 WEEKS; PAMAQUIN DIHYDROCHLORIDE DOSE 2·5 MGM. PER KILO.

FIG. 2. Graphs showing total parasite count in the treated fowl and expected course of the same curve in an untreated infection (logarithmic ordinates). The lower arrows show the times of application of batches of *Aëdes aegypti*, and the corresponding vertical lines the average oöcyst count for each batch.

——— denotes parasites per 1,000 erythrocytes (experimental fowl).
- - - - „ parasites per 1,000 erythrocytes in an average untreated infection.

failed to do so, the average oöcyst counts being reduced to very low values. Both drugs, in doses of 1·5 mgm. per kilo., failed to produce complete sterilization in six hours or at subsequent times up to five days, when the infection had nearly died out. No significant difference, therefore, was detected between the gametocidal activity of plasmoquine (Bayer) and pamaquin (I.C.I.).

Human malaria

Pamaquin tablets (pamaquin methylene di-(2 : 3 hydroxynaphthoic acid)) were given to four adult men. One of these was a normal, healthy individual, another was naturally infected with *Plasmodium falciparum*, and the other two were naturally infected with *P. malariae*. Each individual was treated with three tablets daily for five consecutive days ; one tablet contained the equivalent of 10 mgm. of pamaquin dihydrochloride. The results of this treatment are summarized below :

Case A. Normal individual.
Pamaquin tablets (×3) for five days. No toxic manifestations.

Case B. W. G. Blood : *P. falciparum* ; gametocytes in very considerable numbers. Infection acquired in West Africa.
Pamaquin tablets (×3) for five days.
After three days the crescents began to diminish in number and to alter in appearance ; by the fifth day they had entirely disappeared from the peripheral blood.
On the third day of treatment, however, rings appeared in the blood ; these rapidly increased in number and the patient developed clinical signs of a relapse of his malaria. Two days after the completion of the pamaquin course quinine sulphate, in 10-grain doses thrice daily, was given for three consecutive days, and this cleared the blood of the asexual forms within a couple of days.

Case C. L. E. Blood : *P. malariae* ; asexual and sexual forms in considerable numbers. Infection acquired in West Africa.
Pamaquin tablets (×3) for five days.
By the fourth day of the course the parasites were noticeably reduced in number, and they had completely vanished by the fifth day ; the grosser clinical manifestations of quartan malaria also ceased after this, and did not recur during the ten-days' period of observation before the patient was discharged and put on a course of quinine to forestall relapse.

Case D. G. F. S. Blood : *P. malariae* ; asexual and sexual forms in moderate numbers. Infection acquired in West Africa.
Pamaquin tablets (×3) for five days.
On the fourth day parasites were difficult to find in the blood, and they

had disappeared entirely on the fifth day ; the obvious clinical manifestations of malaria stopped on the same day. During an observation-period of a week there was no relapse, and the patient was then discharged and put on a course of quinine to forestall relapse.

During the pamaquin course, this man complained on three occasions of acute and severe colic for a short time, half to two hours after individual pamaquin tablets. This is one of the recognized toxic manifestations of plasmoquine (Bayer).

A point worthy of note about this case is that he had been seen previously and had been taking quinine, 30 grains on two consecutive days each week for two months. In spite of this, his blood contained parasites, and he was ill and suffering from clinical malaria on his subsequent admission, the last dosage of quinine having been taken only two days previously.

CONCLUSION

The biological action of pamaquin (I.C.I.) has been compared with that of plasmoquine (Bayer). The toxicity of the two preparations is the same, as is also their activity in canary, fowl, monkey and human malaria. These biological tests, therefore, confirm the chemical view that pamaquin (I.C.I.) is identical with plasmoquine (Bayer).

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